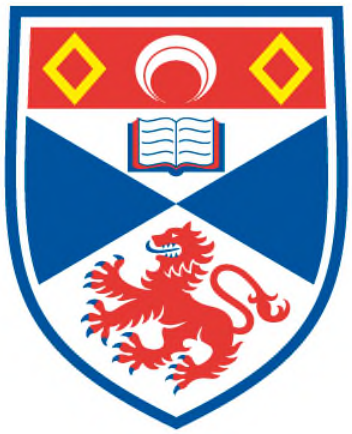
NITRIC OXIDE DONORS FOR THE TREATMENT OF PROSTATE CANCER

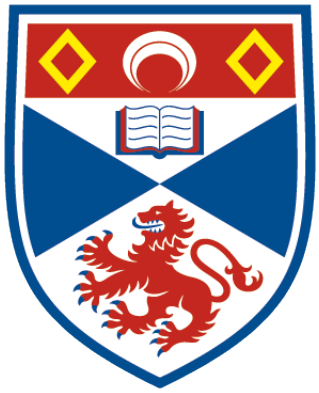
Andrew Nortcliffe

A Thesis Submitted for the Degree of PhD
at the
University of St Andrews



2013

NITRIC OXIDE DONORS FOR THE TREATMENT OF PROSTATE CANCER



University of St Andrews

600 YEARS

School of Chemistry

Andrew Nortcliffe

July 2013

Thesis submitted to the University of St Andrews for the degree of Doctor of Philosophy

Supervisor: Prof. David O'Hagan and Dr Nigel P. Botting

1. Candidate's declarations:

I, Andrew Nortcliffe hereby certify that this thesis, which is approximately thirty thousand words in length, has been written by me, that it is the record of work carried out by me and that it has not been submitted in any previous application for a higher degree.

I was admitted as a research student in September 2010 and as a candidate for the degree of Doctor of Philosophy in September 2011; the higher study for which this is a record was carried out in the University of St Andrews between 2010 and 2013.

Date: _____ Signature of Candidate: _____

2. Supervisor's declaration:

I hereby certify that the candidate has fulfilled the conditions of the Resolution and Regulations appropriate for the degree of Doctor of Philosophy in the University of St Andrews and that the candidate is qualified to submit this thesis in application for that degree.

Date: _____ Signature of Supervisor: _____

3. Permission for electronic publication:

In submitting this thesis to the University of St Andrews I understand that I am giving permission for it to be made available for use in accordance with the regulations of the University Library for the time being in force, subject to any copyright vested in the work not being affected thereby. I also understand that the title and the abstract will be published, and that a copy of the work may be made and supplied to any *bona fide* library or research worker, that my thesis will be electronically accessible for personal or research use unless exempt by award of an embargo as requested below, and that the library has the right to migrate my thesis into new electronic forms as required to ensure continued access to the thesis. I have obtained any third-party copyright permissions that may be required in order to allow such access and migration, or have requested the appropriate embargo below.

The following is an agreed request by candidate and supervisor regarding the electronic publication of this thesis:

Embargo on both all of printed copy and electronic copy for the same fixed period of two years on the following ground: publication would preclude future publication.

Date: _____ Signature of Candidate: _____

Signature of Supervisor: _____

ACKNOWLEDGEMENTS

I would like to start by thanking the late Dr Nigel Botting with whom I started work on this project with. His support and wisdom during the difficult early stages of the work were invaluable in developing my independence as a scientist. It is a great honour to have been part of his research legacy.

Secondly, I would like to thank Prof. David O'Hagan for supervising the continuation of this project in his research group. The creative freedom you have given me has been greatly appreciated, and I will be forever grateful for the support, advice and encouragement over the past two years.

I would like to thank the collaborating scientists on this project, Prof. Jim Ross, Prof. Fouad Habib and Jim Black at the University of Edinburgh; and Dr Ian Fleming at the Aberdeen Biomedical Imaging Centre. Your expertise has been essential for taking the research out of my fume cupboard and moving it towards the real world.

The work in this thesis would not have been possible without the expertise of the technical staff at the School of Chemistry and BSRC. I would particularly like to thank Caroline Horsburgh for mass spectrometry analysis, and Dr Tomas Lebl and Melanja Smith for NMR assistance. I would also like to thank Melanja for her constant support through the ups and downs of my time here in St Andrews.

Many people have made my time here in St Andrews a memorable and enjoyable part of my life. Firstly, I would like to thank Dr Jo Morris for putting up with me as a flat mate for the past two years, and for your friendship, support and advice from the start of

my PhD; secondly, Dr Katy Mould; thank you for being a shoulder to cry on, and a constant source of support, laughs and fun; I have missed sharing a lab with you both, and I wish you both every success in the future. I would like to thank the DOH/NPB group, past and present, for your advice, friendship and knowledge. I would particularly like to thank Dr Neil Keddie and Dr Michael Corr for the proof-reading of this thesis; Alastair Durie and Stephen Thompson for the many chemistry discussions we have had at the whiteboards and Dr Daniel Smith for his continued friendship, support and advice. In addition, I would like to thank Greg Aldred, Lloyd Sayer and Euan Reid for their friendship during the course of my research.

I am also grateful for the friendship and support of the people I have met along the way; Dr Jessica Breen - I suppose I have fluorine to thank for our friendship, thank you for being a constant source of encouragement and advice; Alex "DBC" Rutherford- thank you for the fun and fizz we have had to date, and to many more to come; Dr Matt Smith- the world of social networking started our friendship and the cold hard truth of science continued it, and to Paul Thompson- for being an escape from science to a world of cake, gin and Cher.

And finally, I would like to thank the love and support of my parents, Jayne and Chris; and my brother and sister-in-law, Chris and Caroline. I could not have asked for a more supporting and encouraging family.

Cancer Research UK generously funded the work presented in this thesis.

CONTENTS

I. ABBREVIATIONS

Å	Angstrom
α	alpha
AAT	androgen ablation therapy
Ac	acetyl
ACE	angiotensin converting enzyme
Alloc	allyloxycarbonyl
aq.	aqueous
AR	androgen receptor
Ar	aryl
Arg	arginine
Asp	aspartic acid
bNOS	bacterial NOS
Bn	benzyl
Boc	*tert*-butoxycarbonyl
Br.	broad
brsm	based on recovered starting material
°C	degrees Celsius
calcd.	calculated
Cbz	carboxybenzyl
CDI	*N*,*N*'-carbonyldiimidazole
cGMP	cyclic guanosine-3,5-monophosphate
CNS	central nervous system
CYP450	cytochrome C P450
COSY	correlation spectroscopy
CRPa	castration resistant prostate cancer
CSA	camphorsulfonic acid
CV	crystal violet
DBU	1,8-diazabicyclo[5.4.0]undec-7-ene
DCC	*N*,*N*'-dicyclohexylcarbodiimide
d	doublet
dd	doublet of doublets

decomp.	decomposition
DHT	dihydrotestosterone
DMAP	*N*,*N*-dimethylaminopyridine
DMF	*N*,*N*-dimethylformamide
DMF-DBA	*N*,*N*-dimethylformamide di-*tert*-butyl acetal
DMSO	dimethyl sulfoxide
DNA	deoxyribonucleic acid
dr	diastereoisomeric ratio
δ	chemical shift
EDCI	1-ethyl-3-(3-dimethylaminopropyl) carbodiimide
EDRF	endothelial derived relaxing factor
eNOS	endothelial NOS
ES	electrospray
Et	ethyl
FDR	5-fluoro-5-deoxyribose
Fmoc	9-fluorenylmethoxycarbonyl
g	grams
GABA	γ-aminobutyric acid
Glu	glutamic acid
Gly	glycine
GST	glutathione *S*-transferase
GT	glutathione
G-T	guanine-thymine
GTN	glyceryl trinitrate
GnRH	gonadotropin-releasing hormone
GTP	guanosine-5′-monophosphate
h	hour
HATU	*O*-(7-azabenzotriazol-1-yl)-*N*,*N*,*N*′,*N*′-tetramethyluronium hexafluorophosphate
Hb	haemoglobin
His	histidine
HIF-1α	hypoxia-inducible factor 1α
HMBC	heteronuclear multiple-bond correlation
HOAt	1-hydroxy-7-azabenzotriazole

HOBt	1-hydroxybenzotriazole
HOSu	*N*-hydroxysuccinimide
HPC1	hereditary prostate cancer gene 1
HPLC	high-performance liquid chromatography
HSQC	heteronuclear single-quantum correlation
IC_{50}	dose that results in 50% inhibition
iNOS	inducible NOS
ISDN	isosorbide dinitrate
J	coupling constant in Hertz
L	litre
l	path length
LH	leuteinising hormone
Lys	lysine
M	molar
m	multiplet
m.p.	melting point
m/*z*	mass to charge ratio
MAO	monoamine oxygenase
Me	methyl
MHz	megahertz
min	minutes
mL	millilitres
mtNOS	mitochondrial NOS
mol	moles
NADPH	nicotinamide adenine dinucleotide phosphate (reduced form)
NFSI	*N*-fluorobenzenesulfonamide
NIRF	near infra-red fluorescent
nNOS	neuronal NOS
NOE	nuclear Overhauser effect
NOESY	nuclear Overhauser effect spectroscopy
NOS	nitric oxide synthase
NSAID	non-steroidal anti-inflammatory drug
NMR	nuclear magnetic resonance
NO	nitric oxide

OctR	octreotide
OD	optical density
OG	oxoglutarate
Ox	oxidation
PARP	poly(ATP-ribose) polymerase
Pbf	2,2,4,6,7-pentamethyldihydrobenzofuran-5-sulfonyl
PCa	prostate cancer
PE	petroleum ether
PET	positron emission tomography
PETN	pentaerythritol tetranitrate
Ph	phenyl
Phe	phenylalanine
phen	phenanthroline
π	pi bonding orbital
π*	pi antibonding orbital
ppm	parts per million
PS	polymer supported
PSA	prostate specific androgen
PSMA	prostate specific membrane antigen
PTIO	2-phenyl-4,4,5,5-tetramethylimidazoline-1-oxyl 3-oxide
pVHL	von Hippel Lindau product
PyBOP	benzotriazol-1-yl-oxytripyrrolidinophosphonium hexafluorophosphate
quat	quaternary
q	quartet
R_f	retention factor
RGD	arginine-glycine-aspartic acid
r.t.	room temperature
s	singlet
Ser	serine
sGC	soluble guanylate cyclase
σ	sigma bonding orbital
σ*	sigma antibonding orbital
SNAP	(*S*)-nitrosoacetylpenicillamine
SNP	sodium nitroprusside

SSTRs	somatostatin G-protein coupled receptors
t	triplet
t	tertiary
TBDMS	*tert*-butyldimethylsilyl
tBu	*tert*-butyl
td	triplet of doublets
Tf	trifluoromethylsulfonate
TFA	trifluoroacetic acid
THF	tetrahydrofuran
TLC	thin layer chromatography
TMS	trimethylsilyl
Ts	*p*-toluenesulfonate
T3P®	propylphosphonic anhydride
U	unit (enzyme activity)
Ub	ubiquitin
UV	ultraviolet
Val	valine
w/v	weight per volume

II. ABSTRACT

Chapter One provides a general introduction into the biology and chemistry of nitric oxide, with particular focus on the role of nitric oxide in cardiovascular disease, cancer and hypoxia. It also details the types of organic functional groups used as nitric oxide donors, with detailed discussion of nitrate esters, furoxans and sydnonimines.

Chapter Two discusses prostate cancer. It provides an overview into the development of prostate cancer, prostate cancer staging, and treatment. The key molecular aspects of prostate cancer are detailed, and the types of treatment available outlined.

Chapter Three details the synthesis and activity of NCX-1102, a nitric oxide-donating analogue of the non-steroidal anti-inflammatory drug sulindac, and the synthetic work in the preparation of analogues of NCX-1102, using nitrate esters, furoxans and sydnonimines as nitric oxide-donating functional groups. The compounds prepared were tested against a prostate cancer cell line (PC3) and the cytotoxicity results are presented.

Chapter Four describes the synthesis of nitric-oxide donating analogues of abiraterone, a CYP17 inhibitor for the treatment of prostate cancer. The results of cytotoxicity assays against PC3 cells are detailed.

Chapter Five discusses the application of nitric oxide-donating functional groups in tandem with biologically active motifs. The synthesis of nitric oxide-donating amino acids, and their application to the preparation of nitric oxide-donating RGD peptides and prostate-specific membrane antigen inhibitors is presented, along with representative biological evaluation.

Chapter Six introduces possible future work for the continuation of the project, suggesting the synthesis of fluorinated sydnonimines, prostate-specific membrane antigen inhibitors combined with for prostate cancer imaging and a “tool-box” of nitric oxide-donating bioconjugation reagents.

CHAPTER 1: NITRIC OXIDE

Nitric oxide (NO) is a pleiotropic diatomic molecule, which plays diverse roles in chemistry and biology. It is a radical, with a single unpaired electron in a π^* antibonding orbital (Figure 1). This electron is located on the nitrogen atom (Figure 1). Until 1986, the only known function of NO was as a toxic gas produced from the process of internal combustion in automobiles.[1] However, more recently, this free radical has been implicated in the control of blood pressure,[2] neurotransmission,[3] inhibition of platelet aggregation and immune regulation.[4] It is also implicated in cytostasis and cytotoxicity in cancer,[5] amongst other biological processes.

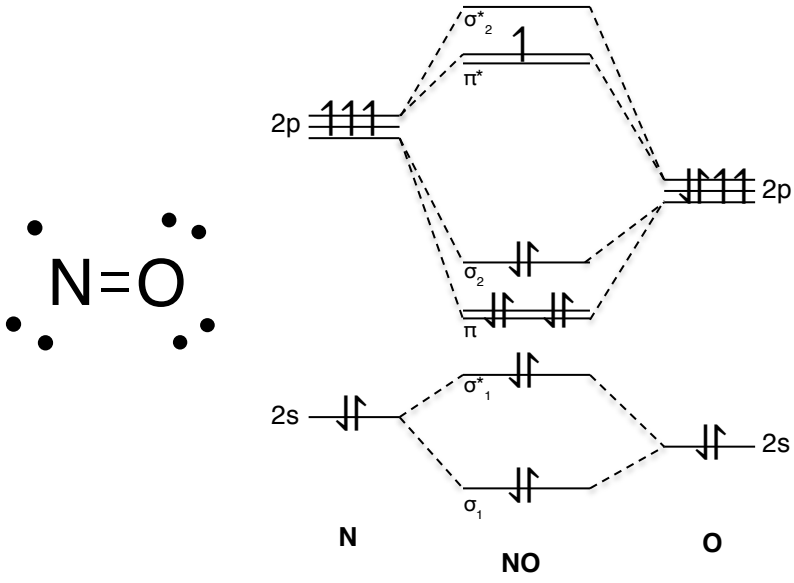


Figure 1: Lewis structure and ground state molecular orbital diagram of NO.

Extensive and diverse research programmes have developed exploring the role of NO and its use as a therapeutic agent. In 1992 the journal *Science* named NO molecule of the year,[6] and in 1998 its role in the relaxation of smooth muscle in the cardiovascular system was the subject of the Nobel Prize for Medicine.[7]

NO is sparingly soluble in water, where a maximum concentration of 2-3 mM can be achieved.[8] It is unstable to many oxidising conditions, and its gaseous native state makes it difficult to introduce into biological systems. As such, efforts have focussed on developing compounds that will release NO through a variety of chemical and enzymatic methods.

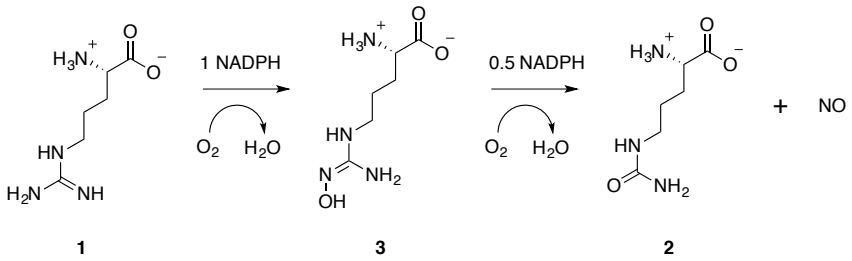
The biosynthesis of endogenous NO is regulated by a series of NO synthase (NOS) enzymes. There are three such synthases,[9] which are characterised by their localisation of expression in cells.

- Endothelial NOS (eNOS) is responsible for generating NO in the endothelium. The endothelium is the layer of cells which forms the inner layer in blood and lymph vessels. eNOS is also expressed in the kidneys, heart and brain, and is the principle NOS enzyme for regulating NO function in the vascular system.
- Inducible NOS (iNOS) is expressed in macrophage cells and areas of the cardiovascular system in an oxidising environment. iNOS has roles in host immunity, anti-microbial properties, and macrophage oxidative burst.
- Neural NOS (nNOS) is found in brain tissues in the central and peripheral nervous system and has roles in cell communication and in interactions with plasma membranes.

Two NOS variants are also known, mitochondrial NOS (mtNOS) and bacterial NOS (bNOS).[9]

The NOS enzymes are responsible for the catabolism of L-arginine (Arg) **1** to L-citrulline **2** (Scheme 1).[9] The guanidine moiety of **1** is hydroxylated to the intermediate *N*-ω-hydroxy-L-arginine **3,** which remains in the enzyme active site. This requires a stoichiometric quantity of oxygen and nicotinamide adenine dinucleotide phosphate in

its reduced form (NADPH). In the second step, *N*-hydroxy-L-arginine **3** is oxidised to the amino acid L-citrulline **2** and NO, upon consumption of oxygen and half an equivalent of NADPH (Scheme 1).



Scheme 1: Pathway for the endogenous biosynthesis of NO.

The endogenous production of NO has diverse roles in biology, acting as a regulator in the cardiovascular system, neurotransmission, inflammation and cell migration. Some these roles of NO summarised below, below with a focus on showing the activity of NO is based on its chemistry.

1.1 BIOLOGICAL ROLES OF NO.

1.1.1 NO IN THE CARDIOVASCULAR SYSTEM, AND ACTIVATION OF SOLUBLE GUANYLATE CYCLASE.

The cardiovascular system is a network of vessels responsible for the distribution of blood, and its components, around the body. Blood vessels are constructed of four main parts as show in Figure 2.[10]

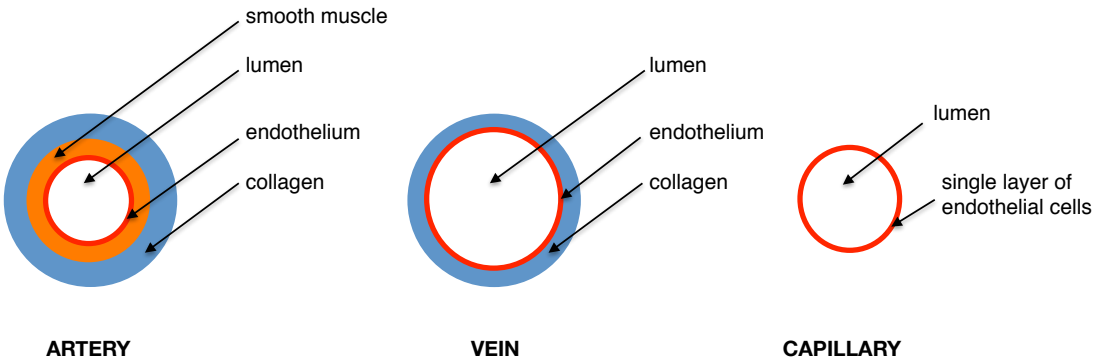
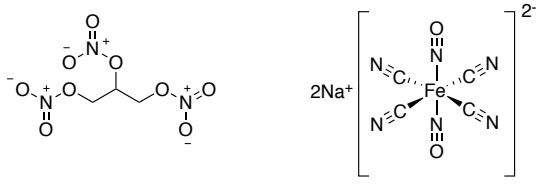


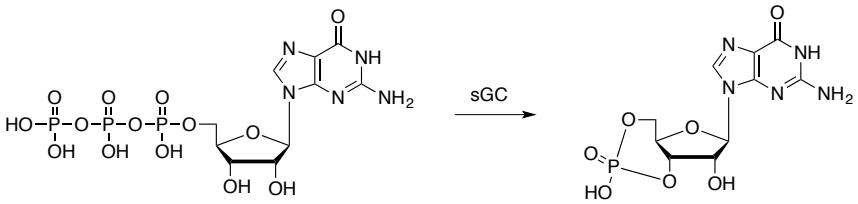
Figure 2: Anatomy of blood vessels.

The interior "hollow" space where blood flows is the lumen, and the interior layer of cells facing into the lumen is the endothelium. Surrounding this in the walls of the vessels are layers of smooth muscle. It is the smooth muscle layer, and its ability to contract and relax, which enlarges the lumen to allow increased blood flow, and *vice-versa* to constrict blood flow. The smooth muscle layer is encased in a layer of collagen. In the cardiovascular system, NO induces the relaxation of the smooth muscle layer.[11] Furchgott identified that relaxation of smooth muscle is not a direct result of acetylcholine signaling, but due to an unknown messenger molecule, which was coined "endothelium-derived relaxing factor" (EDRF).[12] However, the identification of EDRF eluded scientists for many years, as it was produced with a plethora of other products in nanogram quantities in endothelial cells. Glyceryl trinitrate (GTN) **4** and sodium nitroprusside (SNP) **5**, had both been long used clinically for the treatment for angina[13,14] without knowledge of a direct mode of action. It was subsequently elucidated by groups led by Louis Ignarro[15] and Salvador Moncada,[16] that EDRF was NO, and that GTN **4** and SNP **5** were both exogenous donors of NO.[15,16]



4 **5**

The production of NO in the endothelium is responsible for the signaling cascade regulating the conversation of guanosine-5-triphosphate (GTP) **6** to cyclic guanosine-3,5-monophosphate (cGMP) **7**.[17–19] This process initiates a cascade of reactions that results in protein phosphorylation and relaxation of smooth muscle. The transformation of GTP **6** to cGMP **7** is catalysed by soluble guanylate cyclase (sGC) (Scheme 2) and sGC is recognised as a receptor for NO.[20]



6 **7**

Scheme 2: Enzymatic conversion of GTP to cGMP.

sGC is found in the cytoplasm of most mammalian cells, and it is predominantly located in the brain and lungs.[21] sGC is a heterodimer of α and β subunits, the β subunit containing a haem component coordinated to a histidine residue at position 105 (His-105).[22] It has been determined spectroscopically that the iron atom in the haem is in the Fe (II) oxidation state,[23] analogous to deoxyhaemoglobin.[24] The binding of NO to this haem is responsible for activation of sGC. This event is consistent with the coordination chemistry of NO.

NO is a π-acid ligand, analogous to the archetypal co-ordinating ligand carbon monoxide. When CO coordinates to a metal centre a σ bond is formed through overlap between the filled non-bonding orbital containing the lone pair of electrons on CO, and

an empty σ orbital on the metal. In low oxidation state metals, *e.g.* 2^+ or lower, the metal pushes electron density from filled d_{xy}, d_{xz} and d_{yz} orbitals into the empty π*-orbital on CO, in a process described as synergic back-bonding. The overlap of these orbitals results in coordination of CO, with a metal-CO angle of 180° (Figure 3).[25]

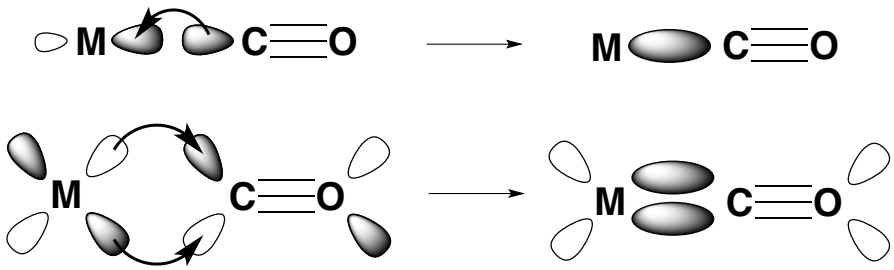


Figure 3: σ and π bonding modes in CO metal coordination.

The ability of a ligand to undergo this type of π-bonding is called π-acidity. Unlike carbon monoxide which has an empty antibonding orbital, NO has one electron in the π*-antibonding orbital, and as such, would be less able to accept a π back-bond. However, NO can lose, or gain electron density as it binds to a metal.[26] As a result, NO co-ordinates between the extremes of NO^+ and NO^-.[26] NO^+ is isoelectronic to CO, and NO^+ coordination is analogous to CO, giving linear complexes with a strong metal-N bond.

In contrast, NO^- contains a half-filled π* anti-bonding orbital and cannot undergo π-back bonding with a metal centre. Instead, it forms a σ-bond from an sp^2 hybridised orbital. The shape of this orbital results in the ligand becoming bent with respect to the metal, at an angle of approximately 120° (Figure 4).[26] This σ bond is still very strong, as NO^- is isoelectronic to molecular dioxygen, which binds to metals in the same way.

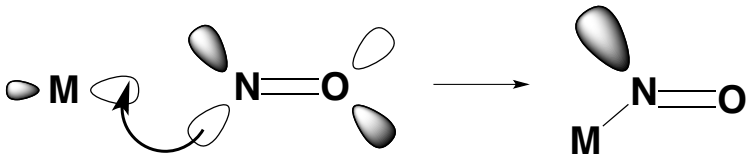


Figure 4: σ-bonding in NO metal coordination

Complexes with linearly bound NO^+ are usually five coordinate, trigonal bipyramidal, with NO in an equatorial orientation,[26] whereas NO^- coordinated complexes are square pyramidal with NO in the apical position (Figure 5).[26]

Figure 5: Geometry of five-coordinate NO complexes.

The ability of NO to coordinate between these two extremes provides the metal with an "electron sink" in the case of NO^+ coordinated ligands. In substitution reactions, a linearly coordinated NO^+ ligand can temporarily accept electron density from a metal centre, becoming a bent NO^- ligand, giving the metal two fewer electrons and allowing it to accept an incoming ligand.[26]

The binding characteristics of NO can be used to explain the biologically observed activation of sGC. As the haem ring is fixed in a square planar geometry, the NO can only bind to give an octahedral complex; NO will bind in bent fashion. However, when NO is bound as NO^-, any ligand *trans* to NO will share a bonding orbital on the metal. There is a very strong donation of electrons *via* the σ-bond from the nitrogen to the metal. However, due to the bent geometry, there is poor overlap between the d-orbitals and the π-orbitals, and the metal cannot redistribute this electron density *via* π back-donation. As such, any ligand *trans* to NO^- would have to donate electron density the

same σ orbital on the metal as NO^- and the coordination would be very weak. When NO binds to the Fe porphyrin, the Fe─His-105 bond is weakened, such that it is enthalpically favoured for His-105 to dissociate, activating sGC. This hypothesis was proven experimentally by Dierks *et al.* through metalloporphyrin reconstitution experiments.[27] By changing the Fe (II) ligand for Mn (II) and Co (II), they demonstrated that the *trans*-effect of the nitrosyl ligand was weakened by Mn (II) and increased by Co (II). This NO coordination and release of His-105 moves the Fe atom out of the plane of the porphyrin, exposing the active site and allowing GTP to enter.[28]

The activation of sGC in smooth muscle is responsible for observed cardiovascular reactions including vasodilation, inhibition of platelet aggregation and activation, inhibition of cell adhesion molecule expression, lipid scavenging, and reduction of tissue factor expression.[29] sGC has been identified as a therapeutic target for cardiovascular disease.[30]

These biological functions have been exploited in medicine to use NO release as a method to modulate diseases such as angina and clotting disorders.[31,32]

1.1.2 NO IN HYPOXIA

Cells which lack sufficient oxygen are described as being hypoxic.[33] In this state, they undergo a series of biochemical responses to compensate. Many cells, such as those found in solid tumours, experience oxygen deprivation in both acute and chronic hypoxic conditions.[34,35] Acute hypoxia arises as a result of poor perfusion to the tumour, and as a result, oxygen delivery is low.[36] Chronic hypoxia develops as a consequence of tumour growth beyond the limit of diffusion from tumour vasculature to cells within the tumour.[37] Hypoxia in the cancer microenvironment is associated with increased invasiveness, metastases, and resistance to radio- and chemotherapy, and is a prognostic

marker for poor clinical outcomes.[37,38] Cells in chronic hypoxic conditions, rather than activating pathways associated with programmed cell death, undergo the "hypoxic response", where the cells adapt to the hypoxic environment and trigger a survival mechanism.[39] The hypoxic response is regulated by expression of Hypoxia-Inducible Factor 1α (HIF-1α).[39] HIF-1α is the only DNA regulatory element truly regulated by oxygen.[40] The hypoxic response is summarised in Figure 6.

HIF-1α is one of two HIF subunits, which when dimerised with HIF-1β form the HIF transcription factor (Figure 6).[41]

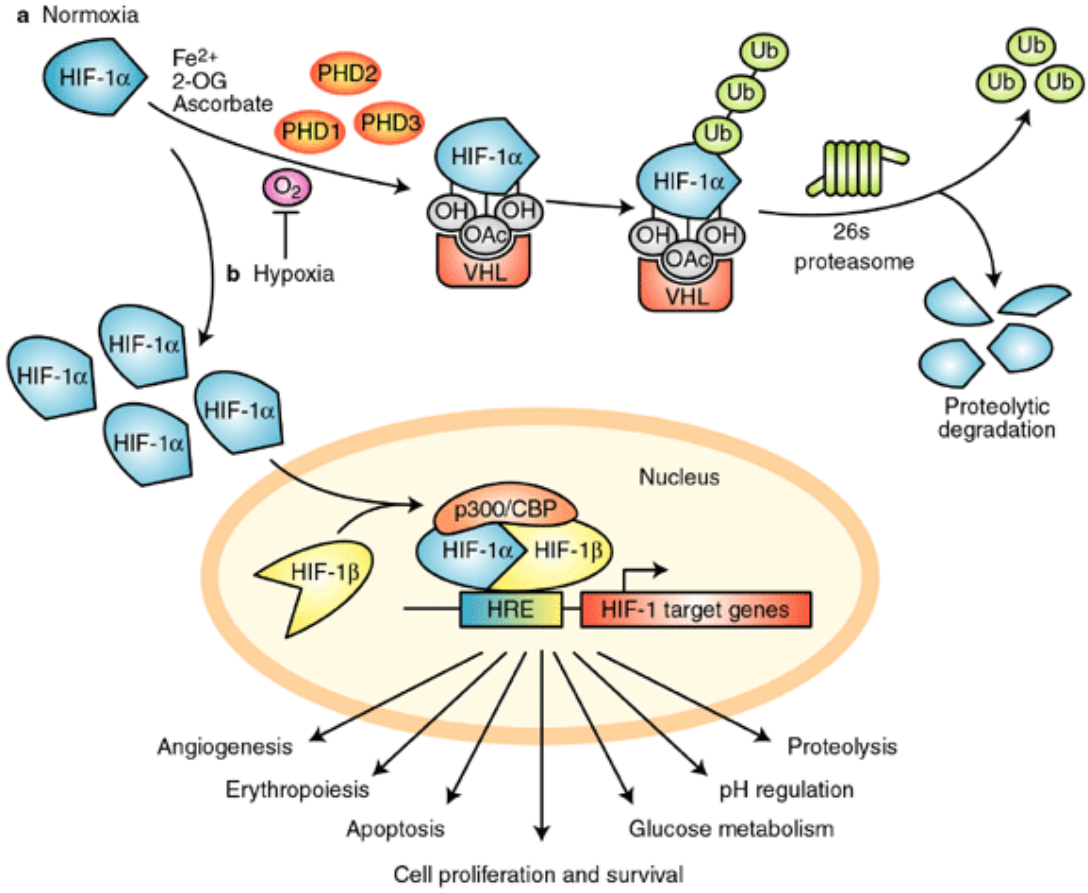


Figure 6: Hypoxia and HIF-1α. The fate of cellular HIF-1α is determined by the oxygen content of the cells (Permissions requested, Carroll and Ashcroft).[42]

Under normoxic conditions, HIF-1α is expressed, but it is rapidly degraded. Two proline residues in the oxygen-dependent domain of HIF-1α are modified by a family of

HIF-prolyl and arginyl hydroxylases.[43] This modified protein then undergoes ubiquitination, mediated by the product of the von Hippel-Lindau (pVHL) tumour suppressor gene, and subsequent degradation.[43] Under hypoxic conditions, the prolyl and arginyl hydroxylase activity is blocked, and as such, HIF-1α is not recognised by the pVHL and accumulates, translocates to the nucleus and interacts with HIF-1β, promoting gene transcription.[44,45] The list of genes activated by HIF-1 is ever expanding, but they can be sub-grouped into classes. Many of the genes are related to adapting to reduced oxygen availability, by increased glucose uptake and glycolysis (GLUT1 and lactate dehydrogenase A).[39] Other genes increase oxygen transport by promoting red blood cell maturation (Epo, transferrin)[46] and angiogenesis (VEGF).[47] HIF-1 also has a role in cell apoptosis and necrosis.[47,48] The combination of hypoxia-induced gene expression, and genetic instability of cancer cells accounts for the observed radio- and chemo-resistance of hypoxic tumours.[38]

It was shown by the group of Salvador Moncada that NO regulates the levels of HIF-1α by two separate mechanisms, one of which is dependent on the mitochondrial electron transport chain.[49] Mitochondria are the major oxygen-consuming components of the cell and it has been reported that regulation of HIF-1α activity is dependent on mitochondrial function.[50,51]

At high concentrations (> 1 μM), NO is responsible for stabilisation of HIF-1α under hypoxic and normoxic conditions; this effect is independent of mitochondrial activity. At low concentrations (< 400 nM), NO prevents the accumulation of HIF-1α in hypoxia, by inhibiting complex IV of the mitochondrial respiration chain.[49] This reduced accumulation of NO is a result of increased degradation of the HIF-1α protein, not by a reduction in HIF-1α synthesis.[49] It was shown that under pathophysiological hypoxic conditions with the addition of NO, molecular oxygen is redistributed from

cytochrome C oxidase in the electron transport chain, as the binding site of O_2 is occupied by NO.[49] This creates a situation where increased O_2 is available for prolyl and arginyl hydroxylases and degradation of HIF-1α.[52]

Reversing the hypoxic response with NO has been identified as a potential treatment for disease states where hypoxia is implicated, such as infant respiratory distress syndrome and neonatal hypoxia respiratory failure.[53–55]

1.1.3 NITRIC OXIDE IN CANCER

In contrast to cardiovascular disease, where the role of NO has been precisely defined in relation to the activation of sGC, the role of NO in cancer is much more diverse.

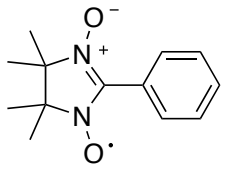
NO has been identified as a cytoprotective, carcinogenic and cytotoxic agent in cancer, depending on the concentration within the tumour. NO has also been used as a cancer therapeutic. There is extensive literature on each subject,[56,57] and some key aspects are discussed in this section.

1.1.3.1 NO CYTOPROTECTION AND TUMOUR PROGRESSION

The role of NO as a cytoprotective agent is closely related to its action as a carcinogen. NO is a free radical, and as such it readily interacts with other free radicals, such as molecular oxygen, and metals in biological systems. Low concentrations of NO have been shown to protect the cells from treatment by peroxides[58,59] by preventing cell injury from lipid oxidation and subsequent endothelial damage.[60] In these systems, NO acts a chain-breaking antioxidant, scavenging peroxide radicals.[60]

Recently, the group of Wogan at MIT reported that endogenously produced NO is a factor in chemoresistance of melanoma cells towards cisplatin treatment.[61] The role of NO in the nitrosylation of the proapoptotic enzyme caspase-3 was proposed as the

mechanism of this cytoprotection.[61] Treatment with PTIO, an NO scavenger, or with *S*-nitrosothiol scavengers reversed the cytoprotection.[61]



PTIO

The over-expression of NOS enzymes and subsequent increased production of NO has been identified as a factor in tumour growth. Malignant glioblastoma are aggressive brain tumours, with limited options for therapy and extremely poor prognosis, with a median survival of under 15 months.[62] Eyler *et al.* reported that increased proliferation of glioma stem cells and tumour growth arises as a result of overexpression of NOS-2, a nitric oxide synthase isoform.[63] Increased iNOS expression has also been examined as a cause of breast cancer metastasis.[64] Large scale studies have revealed that the increased NOS production in breast cancers emerges as a result of upregulation of NOS in the tumour cells, not in the surrounding stromal tissue; and that increased NOS expression was associated with tumour invasion and microvessel density.[65,66] Increased iNOS gene expression is reported in tumours of the brain, head, neck, oesophagus, lung, prostate, bladder, pancreatic and Kaposi's sarcoma.[67]

1.1.3.2 NO AS A CARCINOGEN AND CYTOTOXIC AGENT

NO acts as a carcinogen due to its free radical chemistry and resultant DNA damage. There are three putative mechanisms which have been discussed for how NO induces DNA damage:[68]

(1) Formation of nitrosoamines and DNA base deamination.

(2) Direct modification of DNA by reactive nitrogen/oxygen species.

(3) Inhibition of DNA repair mechanisms.

Nitrosoamines are chemical intermediates associated with nitrosative stress and are potentially carcinogenic.[69] The mechanism of nitrosoamine formation has been proposed based on the gas-phase reactivity of NO.[69]

$$2NO + O_2 \longrightarrow 2NO_2 \qquad (1)$$

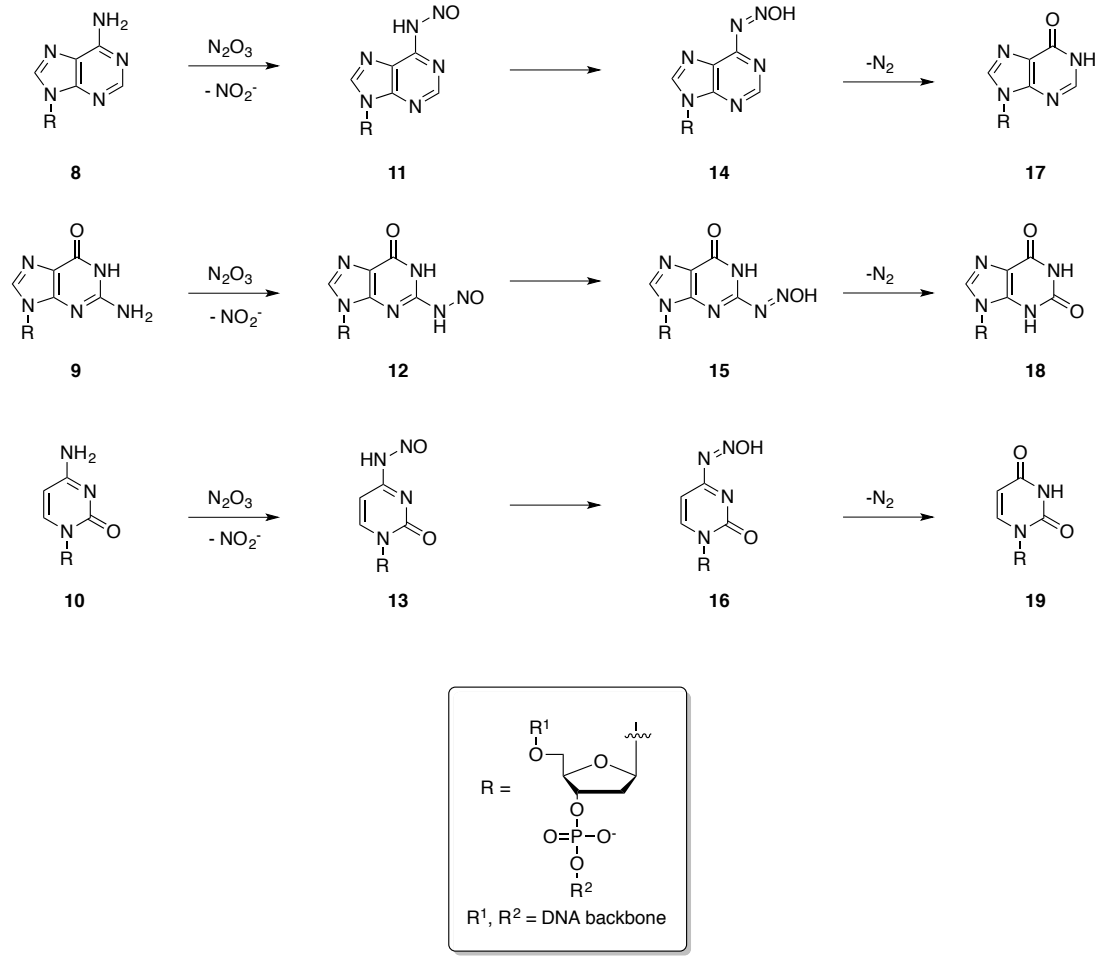
$$NO + NO_2 \rightleftharpoons N_2O_3 \qquad (2)$$

$$2NO_2 \rightleftharpoons N_2O_4 \qquad (3)$$

In the gas phase, NO reacts with O_2 to give NO_2 (1).[69] In an environment with sufficient oxygen, this proceeds to completion and is irreversible.[69] NO_2 is in equilibrium with N_2O_3 (dinitrogen trioxide) (2) and N_2O_4 (dinitrogen tetroxide) (3). N_2O_3 is a potent nitrosating agent. *In vivo*, the primary substrates for nitrosation are the DNA bases adenine **8**, guanine **9** and cytosine **10** (Scheme 3). The putative mechanism for DNA base nitrosation is shown in **Scheme 3**.[68] Nitrosation of exocyclic amines **8-10** is proposed to form primary nitrosoamines **11-13** (Scheme 3).[68] These decompose *via* the *N*-hydroxy species **14-16** with loss of nitrogen to give the deaminated products **17-19** (Scheme 3).[68] Adenine **8** is converted to hypoxanthine **17**, guanine **9** to xanthine **18**, and cytosine **10** to uracil **19**. The conversion of these base pairs results in DNA mismatches in Watson-Crick base pairing as shown in Table 1.

Original Base	*Pairs with*		*New Base*	*Pairs with*
Adenine **8**	Thymine	*Deaminates to*	Hypoxanthine **17**	Cytosine
Guanine **9**	Cytosine		Xanthine **18**	Thymine
Cytosine **10**	Guanine		Uracil **19**	Adenine

Table 1: Products of DNA base deamination.

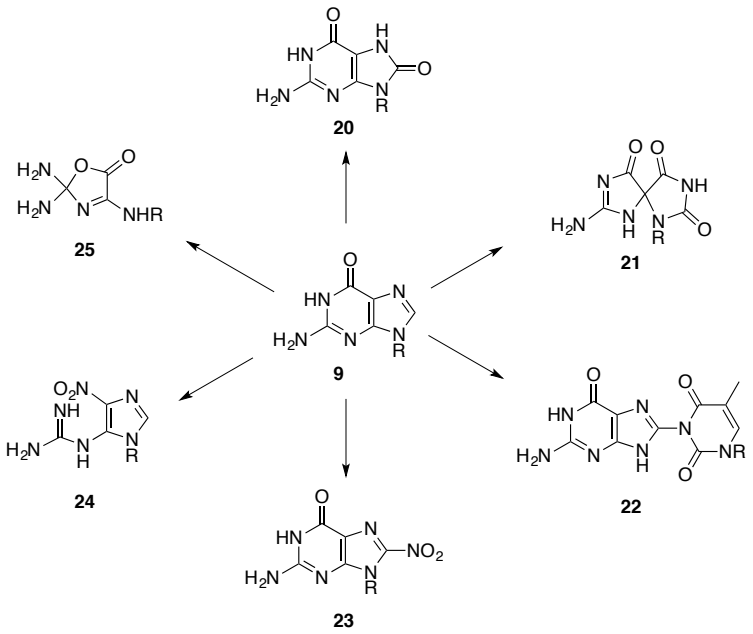


Scheme 3: Deamination of DNA bases by dinitrogen trioxide.

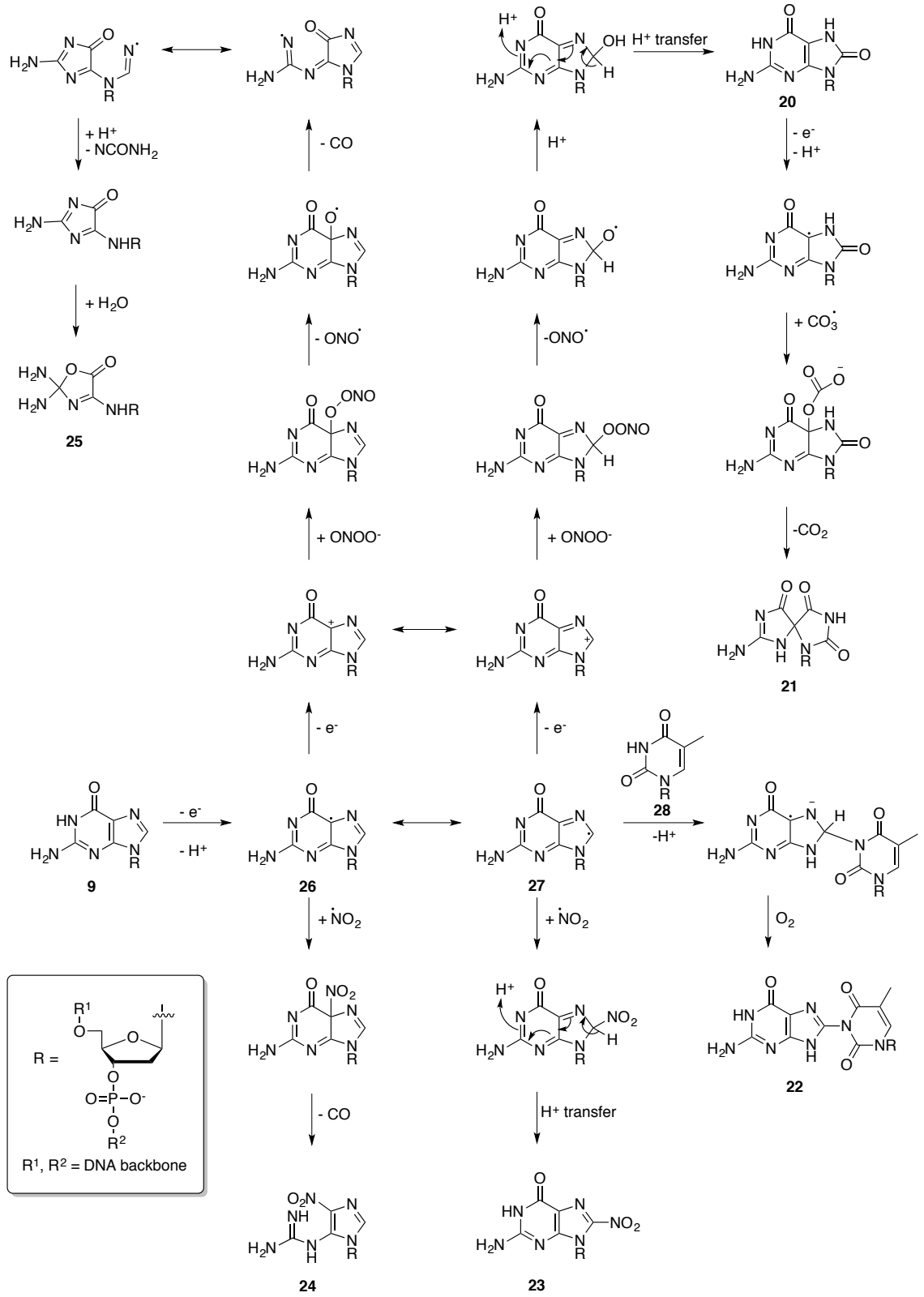
Single strand DNA is more susceptible to this process than double strand DNA.[68] DNA deamination takes place more prevalently in replication and transcription of DNA. In tandem with other genetic mutations these DNA lesions can be carcinogenic. The nitrosation of secondary amines results in the formation of *N*-nitrosoamines which cannot undergo deamination, due to having two alkyl/aryl groups. These are implicated as nitrosyl transfer agents to primary amines. In addition to the deamination of DNA bases, DNA can be directly modified by the action of reactive nitrogen/oxygen species, specifically peroxynitrite ($ONOO^-$).[70]

Superoxide ($O_2^{\bullet -}$) is a common radical in biological systems. *In vivo* superoxide and NO react to give the peroxynitrite ion. This occurs at a rate of 7 x 10^9 M^{-1} s^{-1}, near the

diffusion controlled limit.[71] The reaction of NO with superoxide is faster than the rate of reaction of NO with haem centres, and of superoxide with superoxide dismutase.[72,73] In biological systems, peroxynitrite reacts with carbon dioxide to give peroxynitrosocarbonate ($ONOOCO_2^-$), this decomposes to give $CO_3^{\bullet -}$ and $^{\bullet}NO_2$ radicals, and it is these radicals, along with peroxynitrite which cause DNA damage.[74] The mechanism of peroxynitrite induced DNA damage was exquisitely elucidated by Niles *et al*.[70] Their main finding revealed that the predominant type of DNA damage involved modification at guanine **9** *via* oxidation reactions. The main products were identified as **20**-**25**, summarised in Scheme 4 and 5.[70]



Scheme 4: Summary of main peroxynitrite oxidation products of guanine.[70]



Scheme 5: DNA damage products from guanine.[70]

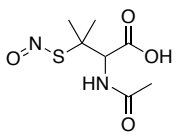
In addition to intramolecular reactions, guanine radical **27** can also react with thymidine bases **28** to result in cross-linked G-T **22**.[74] These DNA modifications, as with deamination, give rise to degraded DNA, which can lead to cancer formation.

Interestingly, the chemistry of peroxynitrite in DNA has been exploited for the treatment of cancers where faulty DNA repair mechanisms have been proposed to lead to genetic instability.[75]

The poly(ADP-ribose) polymerase (PARP) family of enzymes, responsible for DNA repair, is also inhibited by NO. The PARP enzymes contain a zinc finger motif, which is critical for DNA binding.[76] These zinc fingers contain four cysteine residues responsible for binding the zinc atom.[76] Sulfur atoms have a good affinity for NO, and *S*-nitrosothiols are well documented.

Upon exposure to NO, two nitrosative processes can take place.[75] In the first, nitrosation of one of the key cysteine residues by N_2O_3, results in zinc expulsion. In the second, oxidation of a pair of cysteine residues by nitrite or peroxynitrite gives rise to a disulfide bridge, again with release of zinc. This reactivity with zinc finger enzymes has been documented in other enzymes with this DNA binding motif.[77,78]

As described in this section, NO has many different roles in cancer. As a consequence, the development of NO as a therapeutic remains a challenging area of research. In 2004, Jones *et al.* identified that the NO released from (*S*)-nitroso-*N*-acetyl penicillamine (SNAP) **29** affected tumour angiogenesis in a dose-dependent manner.[79]



29

At 26 μM nitrite, angiogenesis was increased by up to 46%; however, a ten fold increase of NO to 281 μM resulted in an 80% inhibition of angiogenesis.[79] In addition

to angiogenesis reduction, a number of kinases were also inhibited at increased NO concentration.[79]

This observation has led to the development of NO-drug hybrids. The combination of a known drug, with an established bioactivity against a therapeutic target, with an NO release functionality, adds an NO "warhead".

This leads us to consider the organic chemistry of NO-releasing functional groups.

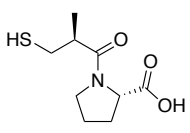
1.2 NITRIC OXIDE DONORS

Since the 1980's, organic chemists have developed a range of NO donors, such as those which release NO spontaneously or donate NO at a controlled rate, and to some extent.[80] There are three main NO-release mechanisms,[80] despite the variety of structural differences between precursor motifs. These are:

1.) Thermal or photochemical decomposition.
2.) Chemical induced release under acidic or basic conditions, or with a metal or thiol.
3.) Enzyme activated decomposition.

The currently developed NO donors, and their mechanism of NO release, is summarised in Table 2.

In addition to molecules which directly release NO, there are many pharmaceuticals which work by an NO-dependent mechanism. A good example is the antiogensin-converting enzyme (ACE) inhibitors such as Captopril.



Captopril

ACE inhibitors inhibit the degradation of bradykinin, a peptide responsible for vasodilation and as a result, lower blood pressure.[81] One of the ways that bradykinin achieves this is by the activation of NOS.[82] The calcium channel blocker, amlodipine, also works by stimulation of NOS.[83] Drugs of this type, in which part of their mechanism of action is the endogenous release of NO are called 'NO stimulators'.[80]

A detailed discussion on the nitrate esters, furoxans and sydnonimines follows, with discussion on their synthesis, biological properties and use in NO-hybrids. These motifs are directly relevant to this thesis.

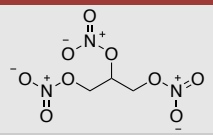
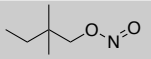
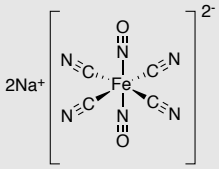
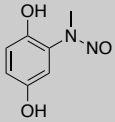
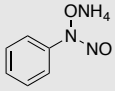
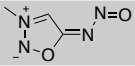
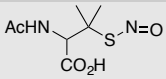
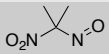
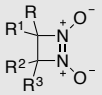
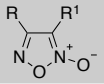
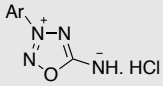
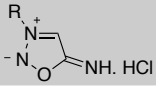
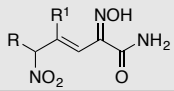
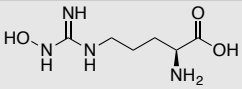
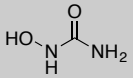
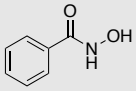
Chemical Class	Representative Compound	Pathway of NO generation	
		Non-Enzymatic	Enzymatic
Organic Nitrate[84]		Thiol	CYP450, GST etc
Organic Nitrite[85]		Hydrolysis, trans-nitrosation, thiol, *hv*, heat	Xanthine oxidase etc
Metal-NO complex[26]		Light, thiols, reductants, nucleophiles	Membrane bound enzyme
***N*-Nitrosamine**[86]		Hydroxide, *hv*	CYP450 enzyme
***N*-Hydroxyl Nitrosoamine**[87]		*hv*, heat	Peroxidases
Nitrosoimine[88]		Thiols, *hv*	Unknown enzymes
Nitrosothiol[89]		Spontaneous, enhanced by thiols, light, metal ions	Unknown enzymes
***C*-Nitroso**[90]		*hv*, heat	Unknown mechanism
Diazetine dioxide[91]		Spontaneous, thiols	Unknown mechanism
Furoxan[92]		Thiols	Unknown enzymes
Oxatriazole-5-imine[93]		Thiols	Unknown enzymes
Sydnonimine[94]		Spontaneous, enhanced by *hv*, oxidants, pH >5	Prodrugs require hydrolysis
Oxime[95]		Spontaneous, O_2/Fe^{III}-porphyrin	CYP450
Hydroxyamine[96]		Auto-oxidation enhanced by metal ions	Catalase/H_2O_2
***N*-Hydroxy guanidine and Guanidine**[97]		Oxidants	NOS, CYP450
Hydroxyurea[98]		H_2O_2/metalloenzyme Haem	Peroxidase
Hydroxamic acid[99]		Unknown	sGC

Table 2: Nitric oxide donors

1.2.1 NITRATE ESTERS

The earliest class of NO donors are the organic nitrates (Figure 7),[84] which are esters of nitric acid with alcohols: GTN **4**, isosorbide dinitrate (ISDN) **30** and pentaerithrityl tetranitrate (PETN) **31** and nicorandil **32** are examples of this class,[84]GTN, ISDN and nicorandil are used clinically.[100]

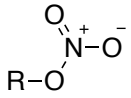
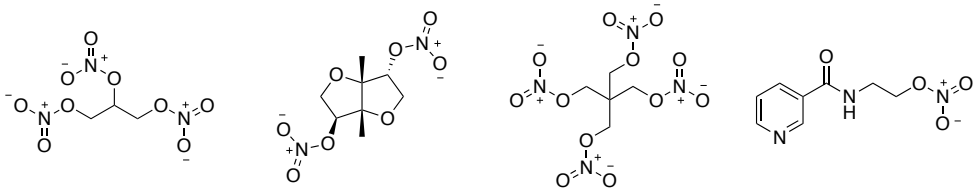


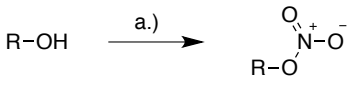
Figure 7: General structure of nitrate esters.



4 **30** **31** **32**

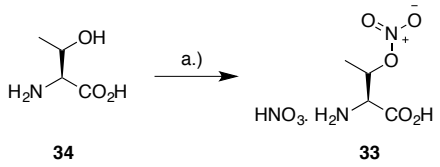
The nitrate ester functional group is widely used in pharmaceuticals,[101] polymers,[102] propellants[103] and explosives.[104] The lower nitrate esters are colourless liquids with a "sweetish" smell,[84] analogous to the corresponding carboxylic acid esters. They are generally insoluble in water. Nitrate esters are generally prepared by one of two methods, these are: esterification of the appropriate alcohol with nitric acid or they are formed nucleophilic substitution on carbon with a source of nitrate.[84]

Alcohol esterification provides the most common method for the preparation of nitrate esters. In general, it involves the use of equal volumes of nitric and sulfuric acid as a nitrating mixture. The combination of these two acids generates the nitronium anion, which upon addition of an alcohol, is quenched to form the corresponding nitrate ester (Scheme 6).



Scheme 6: *Reagents and conditions*: HNO_3, H_2SO_4, 0 °C.

The reaction is carried under cooled conditions as the formation of the nitronium ion and reaction with an alcohol are highly exothermic processes.[105] This method of nitrate ester formation was used by Serkov and Bezuglov in their preparation of the amino acid **33** from L-threonine **34** (Scheme 7).[106]

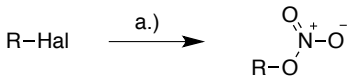


34 **33**

Scheme 7: *Reagents and conditions*: a.) i. HNO_3, CH_2Cl_2, 0 °C, 1 h; ii. Ac_2O, 0 °C to r.t., 45 min, 63%.

While this is an effective method for the preparation of nitrate esters, and uses readily available acids, it is only useful for nitrate esters of acid stable substrates. Complex molecules are unlikely to survive the highly acidic nitrating mixture. As a result an alternative mild method involving nucleophilic substitution has also developed.

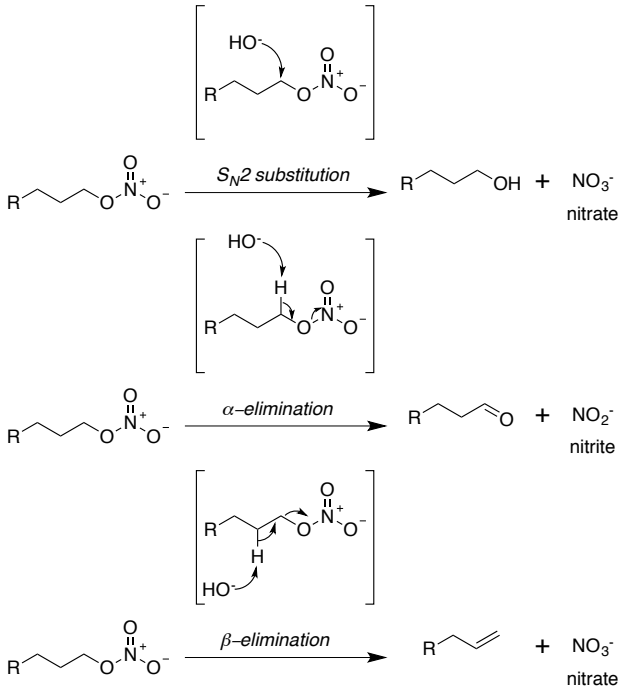
Nucleophilic substitution of an alkyl halide with silver nitrate offers an effective preparation (Scheme 8).[107]



Scheme 8: *Reagents and conditions*: $AgNO_3$, CH_3CN, r.t. to reflux, Hal = Cl, Br, I.

Advantages of this method are neutral reaction conditions and a wide functional group tolerance. In general, the reaction is carried out using acetonitrile as a solvent due to the relatively high solubility of $AgNO_3$, but poor solubility of the silver halide salts that are formed.[108,109] Dry solvent is required to avoid solvolysis of the ester.[110] Alkyl iodides and bromides are generally most reactive under these conditions, and tertiary, allylic or

benzylic alkyl chlorides are also reactive. However alkyl chlorides require pre-activation using a Finkelstein reaction.[108] $NaNO_3$, KNO_3 and Bu_4NNO_3 have also been used in nitrate substitution reactions where the leaving group is mesylate or tosylate.[111–113] Once prepared, nitrate esters are generally stable to most reaction conditions. They are unstable towards strong alkali conditions, and readily undergo direct S_N2 substitution, α-hydrogen elimination of nitrite and β-hydrogen of nitrate, respectively (Scheme 9) with C-O and N-O solvolysis.[110,114–116] The S_N2 reaction has been shown to be predominant.



Scheme 9: Reactions of nitrate esters in aqueous base.

1.2.1.1 NITRIC OXIDE RELEASE FROM NITRATE ESTERS

NO release from nitrate esters has been extensively researched, with a number of mechanisms being proposed.[117] The chemical conversion of an organic nitrate to NO involves a three electron reduction.

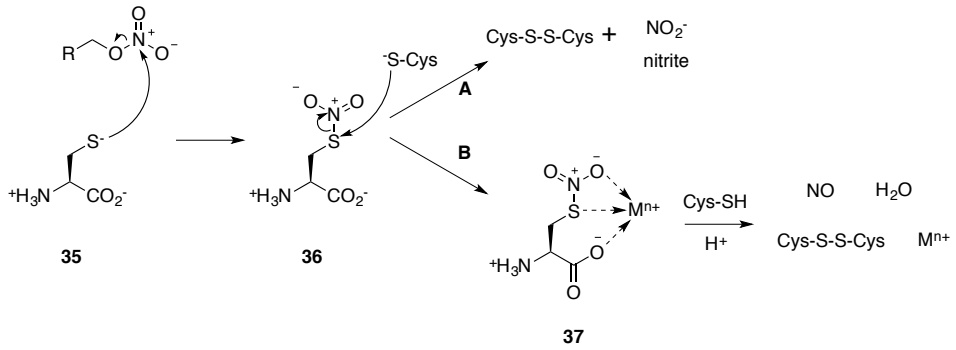
$$RONO_2 + e^- \longrightarrow RONO + e^- + H^+ \longrightarrow ROH + NO_2^- \quad (1)$$

$$NO_2^- + e^- + H^+ \longrightarrow HO^- + NO, \text{ or} \quad (2)$$

$$NO_2^- + M^{n+} \longrightarrow M^{(n-1)} + O^{\bullet} + NO \quad (3)$$

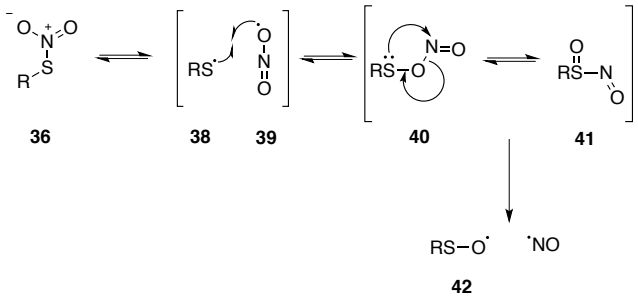
A single electron is responsible for the reduction of the nitrate ester to the corresponding nitrite ester (1). The second, for further reduction and hydrolysis to the alcohol and nitrite (NO_2^-) (1). From here, nitrite can acquire a further electron and a proton to give NO and hydroxide (2), or interaction with a redox active metal M^{n+} resulting in metal oxidation $M^{(n-1)}$ and the formation of NO and an oxygen radical (3). A number of metabolic enzymes appear to be involved in the de-nitrative metabolism of organic nitrates. These include aldehyde dehydrogenase 2,[118] xanthine oxidoreductatase [119] and glutathione-*S*-transferase (GST).[120] No enzyme has yet been proposed to catalyse the three electron reduction of nitrate to NO.[121]

Ignarro suggested that the initial two electron reduction was due to reaction of thiol residues, specifically thiolate cysteine **35** as illustrated in Scheme 10 to generate a thionitrate ester **36**.[117]



Scheme 10: Proposed mechanisms for nitrate ester reduction through a thionitrate ester **36**.

From here, two reaction mechanisms were proposed: The first, (pathway A, Scheme 10), involves the formation of a disulfide bond between thionitrate ester **36** and another cysteine **35**, to liberate disulfide and nitrite. The seconds (pathway B, Scheme 10), involves metal complex **37**, whereby a redox active metal, chelated between heteroatoms to activate the sulfur residue to attack from another cysteine **35**, also generating disulfide and NO.[117] Yeates proposed that the intermediate thionitrate **36** can undergo a radical homolysis of the S-N bond as illustrated in Scheme 11, to give RS$^{\bullet}$ **38** and $NO_2^{\bullet}$ **39**, with the radical localised on oxygen in the latter case (Scheme 11).[122] These combine to give the sulfenyl intermediate **40** and would rearrange to the sulfinyl species **41** (Scheme 11). Radical breakdown of either of these adducts would provide the sulfoxide radical **42** and NO. [122]



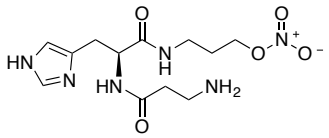
Scheme 11: Proposed mechanism for thionitrate ester decomposition.

Yeates proposed a key role of glutathione-*S*-transferase in the catalysis of these reactions.[122] Formation of the cysteine anion (RS^-) is crucial for the mechanisms proposed, as nitrate esters are poor electrophiles for thiols (RSH) at physiological pH.[123]

1.2.1.2 BIOLOGICAL ACTIVITY OF NITRATE ESTERS

The biological activity of nitrate esters is inherently linked to its NO release properties. Organic nitrates are used in the treatment of a range of cardiovascular diseases such as angina, myocardial infarction, congestive heart failure and the control of blood pressure.[117]

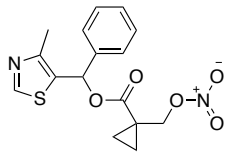
Nitrate esters have been used as an effective way of preparing NO-hybrid drugs. Recent examples include the preparation of carnosine analogues *e.g.* **43**.[124]



43

Analogues such as **43** were able to trap toxic aldehydes and chelate copper acting as NO-donating antioxidants.[124] The preliminary pharmacological activity suggests **43** has a potential for the treatment of complex vascular and neurodegenerative diseases where a reduction of NO availability and oxidative stress is implicated in disease progression.

Qin *et al.* recently developed an "NO-chimera", a molecule containing an NO release mechanism and also acting as a GABA mimetic *e.g.* **44**.[125]

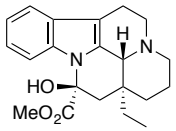


44

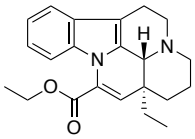
These "nomethiazoles" showed good biological activity in Alzheimer's disease models, citing that the novel scaffold was able to restore both synaptic function and cognition in mice.[125]

Nitrate esters have also been shown to enhance the biological activity of natural products. For example, vincamine **45** is a potent vasodilator,[126] and its semisynthetic derivative, vinpocetine **46** has been used in the treatment of cerebrovascular disease.[126]

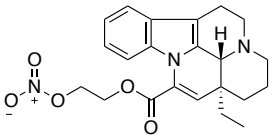
Nitrate ester derivative **47** was shown to significantly increase cerebral blood flow without affecting blood pressure in comparison to vinpocetine **46**.[127]



45



46

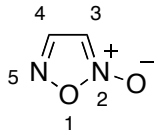


47

1.2.2 FUROXANS

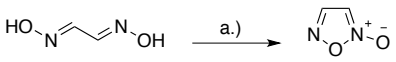
Furoxan is the colloquial name given to the 1,2,5-oxadiazole-2-oxide heterocyclic **48**. First synthesised in the early 1900's,[128] the biological activity of the furoxan was not investigated until the middle of the 20th century. NMR spectroscopy and X-ray crystallography were used to confirm the widely argued structure.[128]

The furoxan structure was first proposed by Wieland in 1908.[129] The position of the *N*-oxide is indicated as 2-, *N*- or N_2-oxide, based on an anticlockwise numbering of the atoms from the ring oxygen (O1), with the pentavalent nitrogen as N2.



48

The unsubstituted 1,2,5-oxadiazole-2-oxide **48** was synthesised in 1994 by Godovikova *et al*. by the action of dinitrogen tetroxide on glyoxime **49** (Scheme 12).[130]



49 **48**

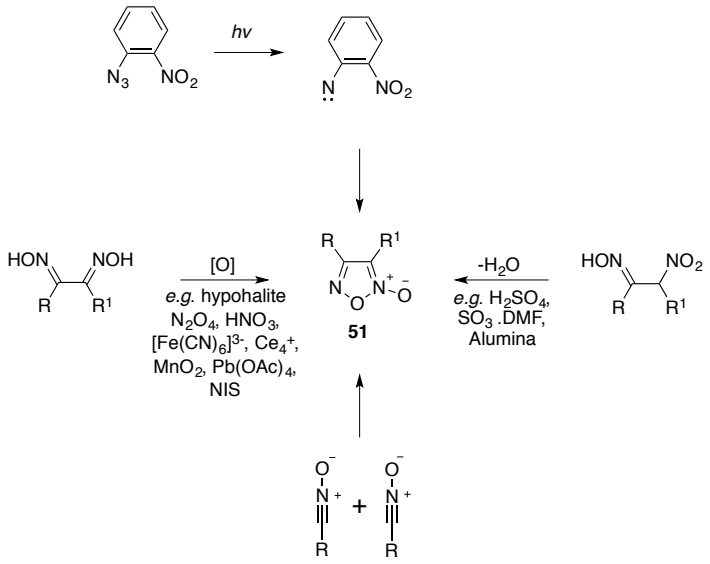
Scheme 12: *Reagents and conditions*, N_2O_4 (1 mol), dichloromethane, 35-38 °C, 45%.[130]

A colourless, stable liquid at room temperature, X-ray analysis shows that **48** is planar and has typical features common to many other furoxans, namely a short N_2-O (exo) bond (1.240 Å) with a corresponding strong IR stretching frequency of 1620 cm^{-1}, the O(1)-N(2) bond is longer at 1.44 Å.[130]

A variety of methodologies exist to prepare furoxans, taking advantage of their potential to substitution reactions at the 3- and 4-positions. Contrary to other *N*-oxide systems (e.g. pyridine *N*-oxide, *N*-methylmorpholine-*N*-oxide), furoxans are not prepared by direct oxidation of the parent 1,2,5-oxadiazole-2-oxide (furazan) **50**.

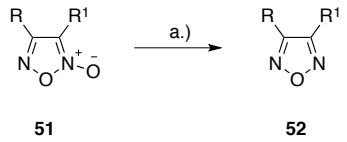
50

The synthetic routes to furoxans **51** vary depending on the substitution required. Commonly used approaches involve cyclisation of a nitro-nitrene intermediates generated from azidonitroolefins,[131,132] oxidative cyclisation of 1,2-dioximes,[133] dehydrative cyclisation of α-nitrooximes,[134] or by nitrile oxide cycloadditions (Scheme 13).[135]



Scheme 13: Synthetic routes to furoxans. R, R_1 = Aryl, SO_2Ph.[108,131,133–135]

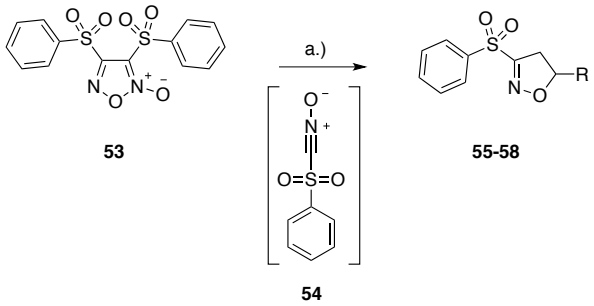
Furoxans *e.g.* **51** undergo deoxygenation with loss of the *N*-oxide to provide the furazan **52** when treated with phosphites (Scheme 14).[136]



51 **52**

Scheme 14: *Reagents and conditions*: $P(OEt)_3$, 150 °C, 5 h, 53%, R = R^1 = Ph.[136]

Furoxans are useful starting materials for a number of chemical transformations, some of which retain the furoxan structure while others utilise the furoxan as a synthetic equivalent of the nitrile oxide for further reaction. For example, Whitney and Nicholas report the use of *bis*(phenylsulfonyl)furoxan **53** as a source of phenylsulfonyl nitrile oxide **54** during the preparation of dehydroisoxazoles **55-58** and isoxazole **59** (Scheme 15, Table 3).[137] The yields were modest, but the products were readily isolated and the starting material was easily prepared as described *vida infra*.



53 **54** **55-58**

Scheme 15: Dipolarophile (1 equiv.), **53** (4 equiv.), xylene, 3-7 h.[137]

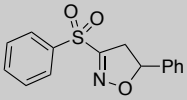
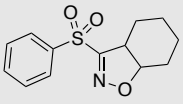
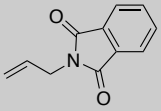
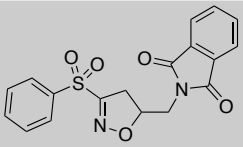
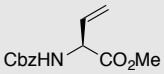
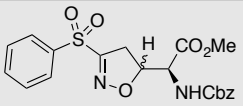
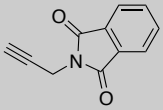
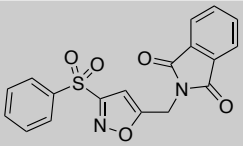
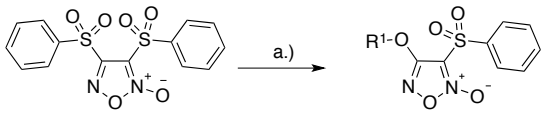
Entry	Dipolarophile	Cycloadduct	Yield
1		 **55**	46
2		 **56**	48
3		 **57**	34
4		 **58**	50
5		 **59**	52

Table 3: Summary of cycloadditions with nitrile oxide surrogate.

Furoxans are deactivated towards electrophilic substitution and are acid (H^+) stable.[128] The furoxan ring is however, is susceptible to nucleophilic attack. Halides, nitro and sulfonyl groups in positions 3 and 4 can undergo nucleophilic substitution. Interestingly, *bis*(phenylsulfonyl)furoxan **53** undergoes substitution with oxygen nucleophiles in the 4-position very selectively due to its increased electrophilicity, to provide furoxan ethers **60** (Scheme 16).[138] The use of functionalised nucleophiles allows the furoxan to be linked to other chemical entities.



53 **60**

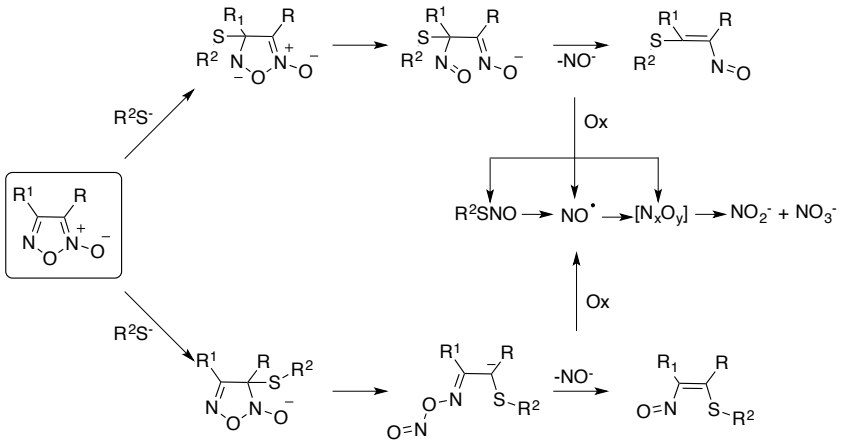
Scheme 16: *Reagents and conditions*: a.) ROH, base (e.g. NaOH, *t*BuOK, DBU). R = alkyl, aryl.

1.2.2.1 NITRIC OXIDE RELEASE FROM FUROXANS

Although the furoxan was first identified in 1903, it was not until 1992 when Feelisch and colleagues reported the release of NO from the furoxan under physiological conditions.[139] With this development, the furoxan presented itself as a bench stable, easily synthesised unit from which to develop new NO donors.[139]

Feelisch determined that NO release from furoxans requires interactions with thiols. In biological systems, the two most prevalent thiol species are proteomic cysteine residues, and glutathione,[139] both of which have been demonstrated in biological tests to initiate the breakdown of furoxans to release NO, with maximum NO release achieved with a 50-fold excess of thiol.[139] The products observed from the reaction of furoxan with an excess of glutathione were nitrite and nitrate, the final oxidation products of NO under aqueous aerobic conditions.[140]

Feelish proposed a mechanism to account for the observed reaction products based on the reaction with thiolate species. The proposed mechanism implies the attack of thiolate onto the 3- or 4-position of the furoxan ring, producing intermediates, which decompose with the release of nitroxyl anion. This can then be oxidised to NO, and in turn, to nitrite and nitrate (Scheme 17).[139]



Scheme 17: Thiolate mediated NO release from furoxans.

Attack of thiolate at the 3- or 4-position is dependent on the appended substituents.[139] The NO-release from furoxans *in vivo,* however, is a much more complicated process. NO-release may be thiol induced, but a possible enzymatic activation cannot be ruled out. This aspect of the furoxan pharmacochemistry has received little attention to date.

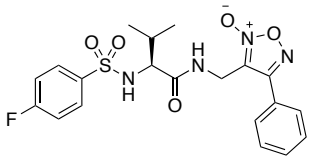
1.2.2.2 BIOLOGICAL ACTIVITY OF FUROXANS.

Furoxan hybrid drugs have been examined and researched extensively and have displayed a wide variety of bioactivities, in many cases greater than the parent drug they are conjugated to.[92] Table 4 summarises a selection of the wealth of furoxan hybrids that have been prepared to modulate the biological activity of a bioactive compound.

Bioactivity	Parent Drug	Study
α_1- antagonists	Prazosin	Fruttero *et al.*, 1995 [141]
β_1- antagonists	Propranolol	Boschi *et al.*, 1997 [142]
Ca^{2+} channel blockers	1,4-Dihydropyridines	Cena *et al.*, 2001 [143]
K^+ channel activators	Nicorandil	Mu *et al.*, 2000 [144]
NSAID conjugates	Aspirin, Ibuprofen	Turnbull *et al.*, 2006; [145], Lolli *et al.*, 2001 [146]
Ca^{2+} channel activators	(*S*)-Isradipine	Visentin *et al.*, 1999 [147]
Anti-H.*pylori*	Metronidazole	Bertinaria *et al.*, 2003 [148]
H_2-antagonist	Lamtidine	Bertinaria *et al.*, 2000 [149]
H_3-antagonists	Imoproxifan	Tosco *et al.*, 2004 [150]
Anticancer	5-Fluoro-2′-deoxyuridine (osteosarcoma, mammary, murine sarcoma)	Moharram *et al.*, 2004 [151]
	Thalidomide (hepatoma, lung carcinoma, prostate)	Wang *et al.*, 2009 [152]
	Pemetrexed (lung carcinoma, gastric adenocarcinoma, hepatoma, leukaemia)	Min *et al.*, 2009 [153]
	Glycyrrhetinic acid (hepatocellular carcinoma)	Lai *et al.*, 2010 [154]
β_2- agonists	Fenoterol	Busonsanti *et al.*, 2007 [155]
Bone resorption inhibition	Bisphosphonates	Lolli *et al.*, 2010 [156]

Table 4: Summary of furoxan hybrid drugs in the literature and the bioactivities they modulate.

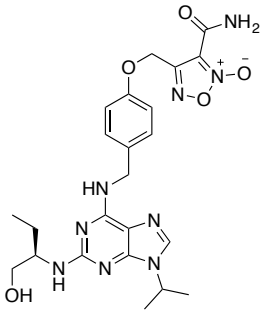
More recently, it was shown by Schiefer *et al*. that NO release from furoxans could be used as a neuroprotective and procognitive agent by activation of the sGC signaling cascade.[157]



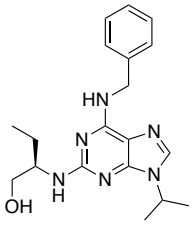
61

Peptidomimetic **61** restored synaptic function to hippocampal slices treated with an oligomeric amyloid-β peptide, the major component of amyloid plaques which impair synaptic function.[157,158] This treatment could have use in the treatment of neurodegenerative diseases such as Alzheimer's disease.

Collaborative research between the groups of Megson and Gasco led to the preparation and analysis of (*R*)-roscovitine analogue **62**.[159] It was shown to be pro-apoptotic for human neutrophils, and that it was more active than the parent (*R*)-roscovitine **63** in termination of the inflammatory response.



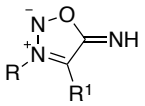
62



63

1.2.3 SYDNONIMINES

Sydnonimines **64** are 5-membered mesoionic heterocyclic rings containing an unusual O-N-N bond motif and an *exo*-imino group.[160]

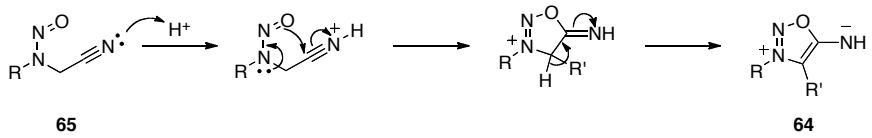


64

Mesoionic is an amalgamation of the terms mesomeric and ionic; and recognises that a mesoionic structure cannot be drawn without the use of a formal positive and negative charge. This distinguishes sydnonimines from other ionic species in organic chemistry such as zwitterions and ylides.[161]

Syndnonimines were first synthesised in the 1950's independently by two groups; Brookes and Walker[160] in London and by Ohta and colleagues in Japan.[162] Both laboratories followed a similar synthetic route involving an acid-catalysed cyclisation of an *N*-substituted *N*-nitrosoglycinonitrile **65**. These were prepared by nitrosation of a *N*-

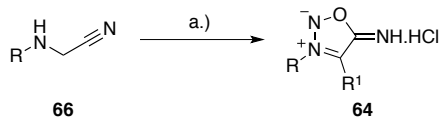
substituted glycinonitrile with nitrous acid (Scheme 18). Protonation of the nitrile by strong acid is followed by concomitant cyclisation followed by elimination of an α-proton to give the sydnonimine **64**.



65 **64**

Scheme 18: Mechanism of formation of sydnonimines.

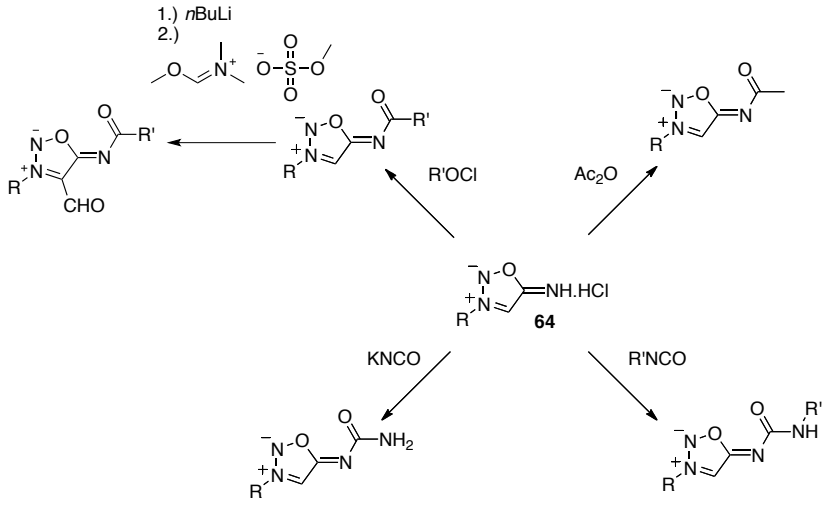
A highly-effective procedure for the synthesis of sydnonimine hydrochloride salts was reported by Turnbull and Beal in 1992.[94] This describes a high yielding (>80%), "one-pot" procedure from an *N*-substituted glycinonitrile. The procedure uses isopentyl nitrite as the nitrosating agent and dry HCl gas to catalyse ring closure (Scheme 19).



66 **64**

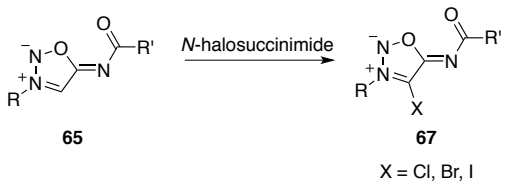
Scheme 19: *Reagents and conditions*: 1.) isopentyl nitrite, Et_2O, 2-12 h, ii.) HCl (g), 5-10 mins, 71-93%, R = alkyl, aryl, R^1 = H.[94]

The preparation outlines the use of isopentyl nitrite as the nitrosation agent in diethyl ether, followed by direct cyclisation catalysed by dry HCl gas. The advantage of this procedure is that the intermediate carcinogenic *N*-nitrosoamine **65**[69,163] species is not isolated. Upon isolation of the sydnonimine **64** further functionalisation can be readily achieved from the *exo*-cyclic nitrogen (Scheme 20) or electrophilic aromatic substitution on the ring. (Scheme 20, 21).[164–167]



Scheme 20: Elaboration of sydnonimine motif **64**. R = Alkyl, Aryl, R_1 = Alkyl, Aryl

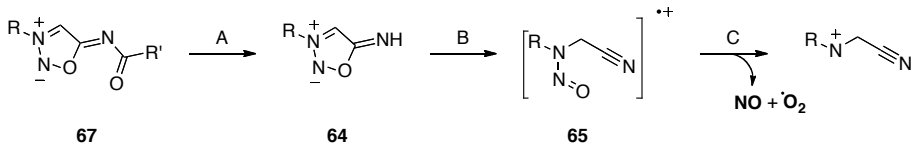
Once a substitutent has been placed onto the *exo*-imino position, then the 4-position of the ring can be substituted. Halogenation with chlorine, bromine and iodine proceeds smoothly using the appropriate *N*-halosuccinimide (Scheme 21).[94,168,169] Halogenation with fluorine has not been reported.



Scheme 21: C4 Halogenation of sydnonimines.

1.2.3.1 NITRIC OXIDE RELEASE FROM SYDNONIMINES

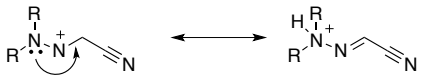
As in the case furoxans, sydnonimines can act as an exogenous source of NO,[140] however, the chemical mechanism of NO release from the sydnonimine moiety is not well understood.[170] The mechanism is believed to begin with the hydrolysis of the *N*-acyl unit of the sydnonimine **67** (Scheme 22, A). [140] probably by an enzymatic process, although chemical hydrolysis has not be ruled out.



Scheme 22: Hydrolysis of sydnonimines to release NO.

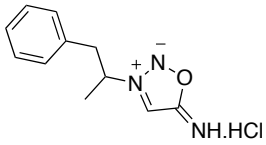
Unsubstituted sydnonimines **64** are unstable as the free base, exposure to oxygen, or enzyme hydrolyses ring opening to give the *N*-nitroso species **65** (Scheme 22, B). which can be oxidised to NO.

Khmel'nitskaya *et al*. studied the mechanism of NO release from a number of sydnonimines by chemical hydrolysis and found that substitution at the 3-position with a dialkylamino group increased NO production ten-fold with a maximum of 31 ± 2% of available NO reported.[171] Alkyl substitututed sydnonimines were poor NO donors under the chemical assay. The working hypothesis proposes that dialkylamino substituents lower the activation energy of NO release by stabilising the cation formed (Scheme 23).



Scheme 23: Resonance stabilisation of dialkylamino substitutents by 1,3-hydride shift. R = alkyl.

Clearly the pathways of NO release *in vitro* and *in vivo* may not be the same.[172] It appears that *in vivo* release is enhanced by enzymatic degradation. For example, the monoamine oxidase (MAO) inhibitor sydnophen **68** with a 1-phenylpropan-2-yl side chain has been shown to be an active NO generator *in vivo*, but in the Khmel'nitskaya assay it showed only 1% NO release.[171]



68

1.2.3.2 BIOLOGICAL ACTIVITY OF SYDNONIMINES

The reported bioactivity for sydnonimines is extensive with the parent heterocycles or minor derivatives, demonstrating anti-tumour,[173] anti-inflammatory[164] and spasmolytic (muscle relaxant) activity.[174] For example, Yashunskii *et al*. reported an extensive number of 3- and 4-substituted alkyl, aryl and aralkyl sydnonimines demonstrating reversible inhibitory properties of MAO and peripheral sympathomimetic CNS activation.[175]

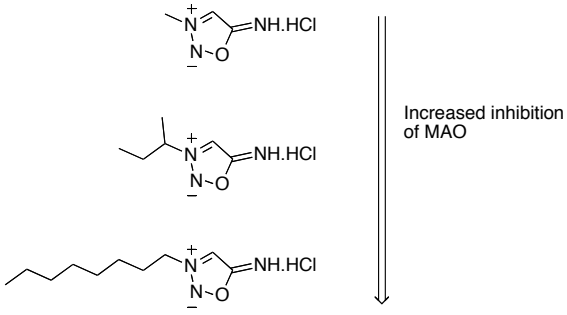
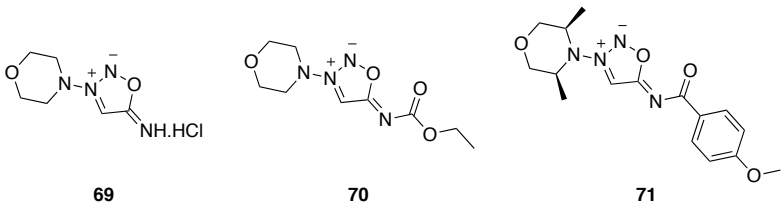


Figure 8: Increased inhibition of MAO of alkyl chain sydnonimines.

Sydnonimines have shown significant promise as cardiovascular therapeutics. These are exemplified in three key compounds, SIN-1 **69**, Molsidomine **70** and CAS-936 **71**.

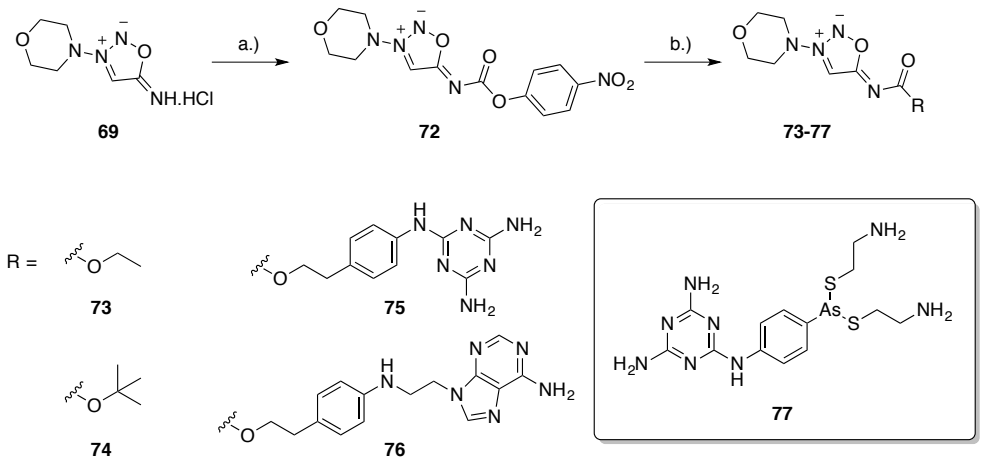


69 **70** **71**

Molsidomine **70** is an antihypertensive, long acting vasodilation drug used clinically for the treament of stable, moderate angina pectoris. It is a prodrug for SIN-1 **69** and derives its activity from the release of NO.[176] CAS-936 **71** is structurally similar to

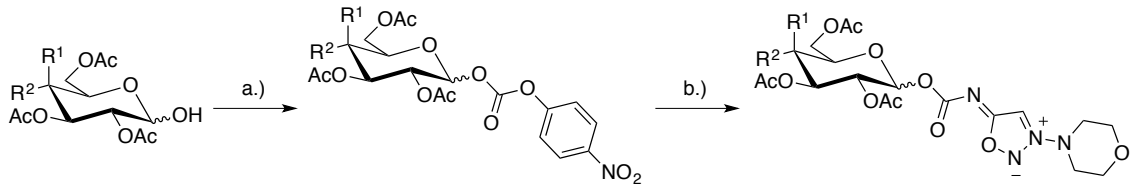
molsidomine **70** and acts as an antiischemic agent. However, while molsidomine **70** requires liver metabolism to generate its active component, CAS-936 **71** is believed to possess a vasodilating activity of its own. CAS-936 **71** is not used clinically.[177]

Soulère *et al*. developed a series of sydnonimines **73**-**77** as potential trypanocidal agents against *Trypanosoma equiperdum*,[178] with compound **75** showing improved activity compared to cymelarsan **77**[178] an arsenic based drug for the treatment of African trypanosomiasis (Scheme 24).[179]



Scheme 24: *Reagents and conditions*: a.) 4-Nitrophenyl chloroformate, pyridine, 25 °C, 16 h, 70%; b.) **73**, EtOH, reflux, 3.5 h, 83%; **74**: *t*-BuOH, reflux, 18 h, 70%; **75**: 2,4-[(4,6-diamino-1,3,5-triazin-2-yl)amino]phenyl-1-ethanol, CH_3CN, 82 °C, 4 h, 26%; **76**: 2′,3′-*O*-isopropylidene-adenosine, CH_3CN, 4 h, 50%.[178]

Cai *et al.*[180] developed a series of glycoside based sydnonimines **82**-**83** (Scheme 25). These carbohydrate conjugated sydnonimines could be deprotected to give the parent sugar. NO was catalysed by the action of glycosidases.[180]



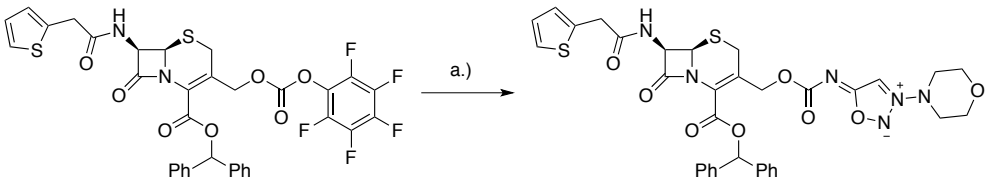
78, R^1= H, R^2= OAc
79, R^1= OAc, R^2= H

80, R^1= H, R^2= OAc
81, R^1= OAc, R^2= H

82, R^1= H, R^2= OAc
83, R^1= OAc, R^2= H

Scheme 25: *Reagents and conditions*: a.) 4-Nitrophenylchloroformate, Et_3N, CH_2Cl_2, r.t., 4.5 h, **80** = 90% , **81** = 88%, (1:3 α/β); b.) **69**, pyridine, r.t., 12 h, **82** = 35% (α), 10% (β), **83** = 40% (α+β).[180]

Perflurophenyl carbamate **84** was also successful for the synthesis of active esters of β-lactam cephalosporins (Scheme 26).[181] Coupling of **84** with SIN-1 **69** furnished sydnonimine **85** in a 4:1 inseparable mixture of 3'- and 2'- position isomers in 42% yield (Scheme 26). The release of NO from **85** was catalysed by penicillinase.[181]



84 **85**

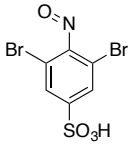
Scheme 26: *Reagents and conditions*: a.) **69**, pyridine, 42%.

1.3 MEASURING NO RELEASE.

Measuring the concentration of NO in samples of air or biological milieu is difficult. The rapid oxidation of NO to nitrite (NO_2^-) and nitrate (NO_3^-) by oxygen, and its reaction with superoxide to give peroxynitrite ($ONOO^-$) results in a very short half-life for NO. A number of assays have been developed measure NO, however, they are indirect assays generally measuring oxidation products or NO-adducts.

NO is a radical and EPR spectroscopy can be used to detect its presence. However, the direct detection of NO directly by EPR is not possible, as the relaxation time of the stimulated electron is too rapid to be detected.[182] In order to detect NO by EPR, nitroxide spin traps such as 2-methyl-2-nitrosopropane **86** or 3,5-dibromo-4-nitrosobenzenesulfonic acid **87** are required.[183]

86



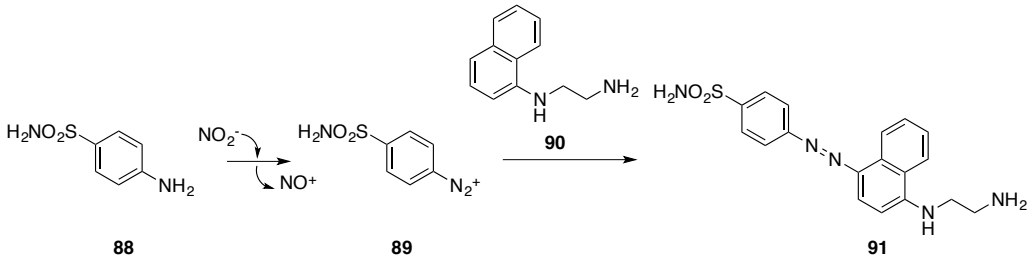
87

Haemoglobin (Hb) has also been used as a spin trap where the nitrosyl-Hb adduct is readily measurable by EPR.[184] This method has been used to detect NO production from arterial rings[184] and for the investigation of the production of nitrosyl-Hb in septic shock.[185,186]

Hb can also be used to detect NO spectrophotometrically when it reacts with oxohaemoglobin ($Hb(Fe^{2+})O_2$) to give nitrate and methemoglobin ($Hb(Fe^{3+})$)[15] the latter can be observed at 406 nm.[15] The reaction of NO with Hb occurs in less than 100 ms,[187] and as such it is useful for determining the production of NO from a biological sample over an extended period. The disadvantage of this technique is commercially available haemoglobin must first reduced be with dithionite, and purified by gel chromatography.[187]

A second widely used spectrophotometric analysis is the Griess test.[188] It is generally accepted that the complete exclusion of oxygen from biological samples or buffers is virtually impossible, and that any oxygen present will rapidly oxidise NO to nitrite, and then from nitrite to nitrate. The oxidation of nitrite to nitrate is much slower, and as such, there is a build up of nitrate. Griess developed a diazotisation method for the

detection of nitrite, and this has been adopted as a proxy measurement for the production of NO (Scheme 27).[188]



Scheme 27: Reactions of the Griess test for nitrite determination.

In this assay, a sample is incubated with sulfanilamide **88** in the presence of an acid, usually phosphoric acid. This generates the nitrosonium ion, which reacts with sulfanilamide **88** to give diazonium **89**. Following incubation, *N*-(1-naphthyl)ethylenediamine **90** is added, and the sample is further incubated. During this time, coupling between the diazonium **89** the amine **90** generates the diazo compound **91** that can be detected spectrophotometrically between 550 and 570 nm. The Griess test is the most widely used assay for detection of NO. It is easy to perform, and the materials required are commercially available in kit form. The method can be adapted such that NO release can is assessed in samples in 96-well plate format using a microplate reader, allowing for high-throughput analysis of NO production.[188] In addition to the detection of NO by spectrophotometric methods, NO can be detected by chemiluminescence. The chemiluminescence assay was first developed to assay the concentration of NO in atmospheric samples.[189] NO reacts with ozone to generate light ((1) and (2)), which can be detected by a sensitive photomultiplier tube.

$$NO + O_3 \longrightarrow NO_2^{\bullet} + O_2 \qquad (1)$$

$$NO_2^{\bullet} \longrightarrow NO_2 + h\nu \qquad (2)$$

This assay can be modified to detect NO in solution by driving solubilised NO into the gas phase by bubbling helium through the solution. The chemiluminescence assay detects NO production to a level of 10^{-13} M (100 fM).[189] This technique detects oxygen metabolites of NO through the use of an acidifircation step; however this process can result in an over-estimation of NO levels.[190]

CHAPTER TWO: PROSTATE CANCER

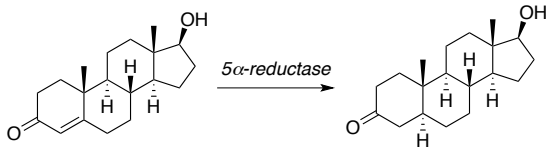
In the United Kingdom, prostate cancer (PCa) accounts for 13% of male cancer deaths and represents 25% of all new male cancers diagnosed. In 2010, this equated to 40,975 new cases, which is approximately 134 cases per 100,00 men in the UK. PCa commonly affects men over the age of 50, and as a consequence presents significant challenge in diagnosis and prognosis for clinicians, as progression from a localised tumour to metastatic disease can be rapid, and currently there are no tumour markers known to identify aggressive forms of the disease.

2.1 THE PROSTATE

The prostate is an exocrine gland of the male reproductive system. It is found in the majority of mammals. In humans the prostate is found in the lower abdomen and surrounds the urethra. Anatomically, the prostate is divided into sections, described as zones or lobes. It consists of epithelial glands and is encased in a fibromuscular stroma; the prostatic capsule.[191]

The role of the prostate is to store and secrete prostatic fluid, a component of semen. These secretions are alkaline and are generally composed of basic sugars, zinc and proteolytic enzymes. Prostatic fluid also contains prostatic acid phosphatase and prostate-specific antigen (PSA); both of which are elevated in PCa and are used as diagnostic markers.[192]

The prostate is regulated by specific androgens produced by the body.[193] The main regulatory androgen is testosterone **92**, a C19 steroid produced in the testes under the control of pituitary hormones.[194]



92 **93**

Testosterone **92** is then converted into dihydrotestosterone **93** (DHT) by the action of 5-alpha reductase. DHT can then act through the androgen receptor (AR) in prostate tissue.[195]

2.2 PROSTATE CANCER

Tumours of the prostate have been shown to develop over 20-30 years or more, and up to 10% are based on an inherited genetic predisposition to prostate tumourigenesis.[196] Post-mortem data estimates that approximately 50% of all men in their fifties have histological evidence of PCa. This rises to 80% by age 80.[197]

PCa arises in the epithelial or progenitor cells within the prostate and is initially an adenocarcinoma.[198] The discovery of the requirement for androgens for PCa growth was the topic of pioneering work by the Nobel Prize (1966, Physiology or Medicine) laureate Charles Huggins.[198]

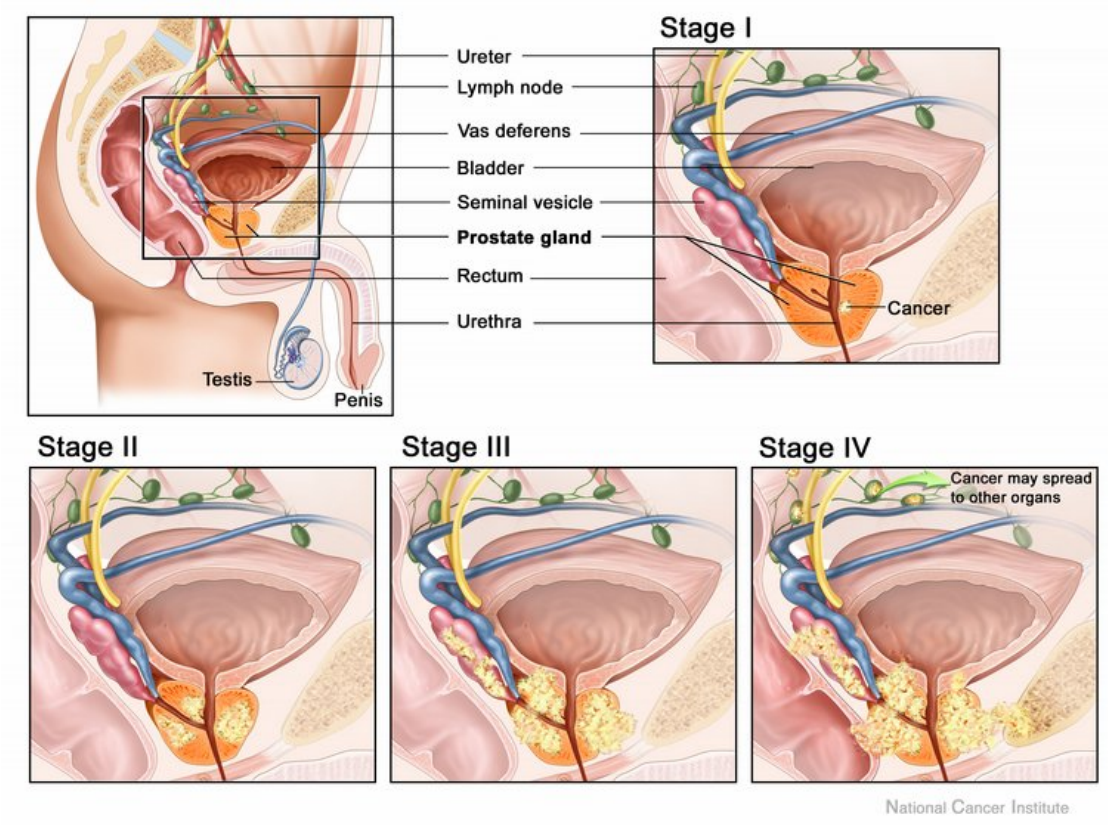


Figure 9: Pathology of prostate cancer (Image from National Cancer Institute Library)

Stage I of PCa involves the accumulation of malignant cells within otherwise normal prostate glands (Figure 9). Stage I is regarded as a carcinoma *in situ*. Due to the complex biochemistry within the tumour, genetic markers to gauge the innate malignant potential of the tumour, at this stage, have yet to be identified.[199–201] Diagnosis of PCa at Stage I rare as the tumour presents only in small pockets within the prostate, and cannot be identified by a digital rectal exam.

Further growth of the tumour to the point it can be palpated upon examination, but remains within the gland, is characteristic of a Stage II carcinoma. At this stage, the tumour may have spread bilaterally to both lobes of the prostate (Figure 9).[202] Further diagnosis of the tumour is based on a patients PSA level, the rate of change of PSA level and the Gleason score; a measurement of cell abnormality based on histology.[203] A combination of these results provides critical information to clinicians regarding

anticipated tumour behaviour. Up to this point, the PCa can still be treated surgically; this is discussed in detail (*vida infra*).

Once the tumour advances through the prostatic capsule, the cancer has reached stage III. Stage III tumours are associated with metastases that remain within the pelvis (Figure 9).[202] From Stage III onwards there is a marked change in overall patient prognosis. Metastases further than the prostate represent Stage IV (Figure 9). Prostate metastases are found in the bones in up to 80% of cases,[204] with lung, liver, pleural and adrenal metastasis also common.[205] Post-mortem analysis has indicated that metastasis occurs through the spinal veins, and through the vena cava.[205] In general, treatment at this stage with androgen ablation therapy is effective in the majority of patients, however, they experience disease recurrence in a median of 12-18 months. This represents Stage IVa of prostate cancer progression, termed castration-resistant prostate cancer (CRPa). At this advances stage, tumour growth is independent of androgens and treatment options are limited, metastases are extensive and the prognosis is poor.

2.3 GENETIC PREDISPOSITION TO PROSTATE CANCER

Familial studies on PCa have consistently shown a strong hereditary component in PCa risk, greater than any other type of cancer.[196] Men with PCa were more likely to report having a brother or father with PCa than the family of their spouses.[206] An early study on the molecular genetics of familial PCa identified a chromosomal region, which has gone on to be named the hereditary prostate cancer gene (HPC1).[207] However, further studies into the effect of the HPC1 gene on PCa have produced ambiguous results.[208–211] To date, no oncogene has been linked conclusively with the initiation or early progression of PCa.[212] However, a number of classical oncogenes have been researched in PCa to further understand the molecular progression of carcinogenesis and the

clinical impact on prognosis.[213] Most recently, it was identified that germline mutations in *BRCA1/2* are responsible for more aggressive PCa phenotypes, distant metastases and a higher probability of lymph node involvement,[214] with overall poor patient prognosis. This suggests that the *BRCA1/2* genes could be used as prognostic markers for PCa in the same way they are used in breast and ovarian carcinoma.[215,216]

While the number of individual mutations examined are too great to describe in detail, a paradigm of progression has been established, which correlates the most common molecular changes in PCa throughout its growth (Figure 10).[217]

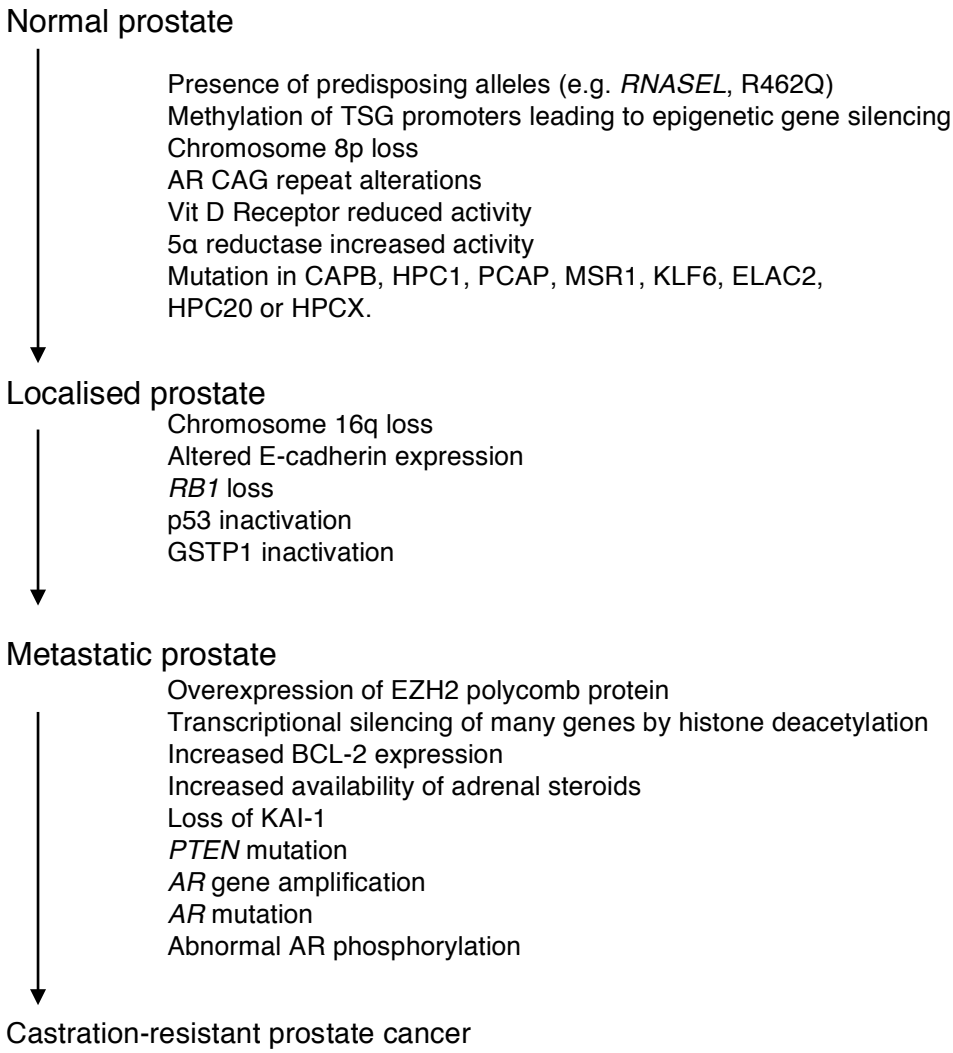


Figure 10: Molecular progression in PCa (adapted from Karayi)[217]

2.4 MOLECULAR FEATURES OF PROSTATE CANCER

A number of key molecular architectures in PCa have been used to identify the molecular mechanisms at play during its progression, and have been identified as targets for treatment and diagnosis. Detailed below are key molecular features which contribute to the progression and treatment of prostate cancer.

2.4.1 THE ANDROGEN RECEPTOR

The androgen receptor is a nuclear receptor responsible for the binding of testosterone or dihydrotestosterone (Figure 11).[218] It is composed of amino-terminal and carboxy-terminal ligand binding domains, and a DNA-binding domain containing two zinc fingers.[219,220]

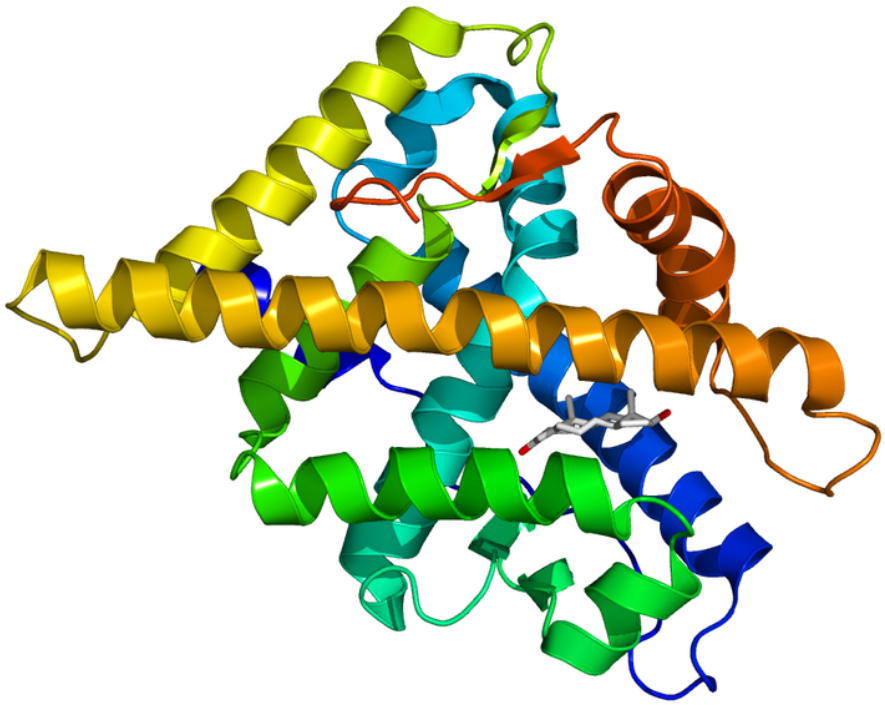


Figure 11: Crystal structure of the androgen receptor (rainbow helices) complex with testosterone (white sticks). Created using PyMol based on PBD crystallographic structure 2AM9.[219]

Like other DNA-binding proteins, it is chaperoned by heat shock proteins when free from a ligand.[221]

The main function of the androgen receptor is as a DNA-binding transcription factor. Upon ligand binding, the AR sheds its molecular chaperones and translocates to the nucleus, it then dimerises and results in regulation of androgen response genes. The AR also interacts with other proteins in the nucleus to regulate gene transcription.

Male development is dependent on the action of the androgen receptor.[222] After puberty, the androgen-receptor is responsible for the regulation of genes related to male sexual characteristics, and for growth-quiescent maintenance of the prostate epithelial tissue (where the AR is widely expressed) and for increased production of PSA.

Prostate epithelial tissue is the main tissue type which is transformed into prostate adenocarcinoma.[223] Genetic faults in these growth-quiescence elements results in PCa progression which is regulated by the supply of androgens within the cell. The act of androgens alone interacting with the androgen receptor alone does not appear to promote carcinogenesis in humans.

2.4.2 ANDROGEN RECEPTOR IN PROSTATE CANCER

The AR is heterogeneously expressed throughout primary prostate tumours, and androgen receptor expression has no correlation with a patient's response to androgen ablation therapy.[224] Anti-androgen therapy directly blocks the activity of the AR, and consequentially begins the cascade to induce apoptosis.[225] While AR inhibition is effective in treatment at Stage IV of the disease, the AR is implicated in the development of CRPa. In most cases of CRPa, the expression and activity of the androgen receptor are maintained,[226] and the receptor drives the progression of the

disease in castration-levels of testosterone.[227] A number of mechanisms have been suggested for this activity.

The first is a hypersensivity pathway. This mechanism suggests that the prostate cancer has a lower threshold for androgen activity and can be activated in castration-levels of testosterone.[228] One postulated mechanism is the overexpression of the AR gene.[229] AR overexpression results in increased sequestration of the remaining low levels of testosterone, allowing growth at castration-levels of testosterone. Approximately 30% of CRPa tumours overexpress the AR after androgen ablation,[230] although mutation may be independent of selective pressure of androgen blockade.[231] Amplification can be a result of clonal selection of the cells which will proliferate in the low levels of androgen.[232] The hypersensitivity pathway has also shown that local production of DHT can account for proliferation in CRPa.[233] Increased 5a-reducatase activity may account for the observed increased levels of DHT in castration-levels of testosterone.[195]

The second pathway has been coined "the promiscuous pathway".[228] Somatic mutations in the AR gene result in mutations in the AR binding site, such that it can be activated by other ligands such as non-androgens,[234–236] antiandrogens,[237,238] or in the absence of a ligand.[239] Genetic changes can also result in overexpression of the androgen receptor itself, either in a wild type, or mutant form. A study by Shi *et al*. examined the function of the androgen receptor in CRPa tumours.[240] The results are shown in Table 5. In summary, they found that 77% of tumours had AR activity at partial or increased levels to wild type.[240]

Androgen Receptor Activity	Percentage
Loss of function	16
Wild Type function	7
Partial function	32
Gain of function	45

Table 5: Androgen receptor function in CRPa tumours.[240]

In the absence of AR mutation, CRPa may be driven by ligand-independent mechanisms. Such pathways have been classed as "outlaw pathways". Outlaw pathways are the induction of AR activity through androgen-independent mechanisms. AR activation by growth factors,[239] receptor tyrosine-kinases (*e.g.* HER-2/neu)[241] and aberrant Akt signaling[242] have been identified in the literature.

These examples show that the AR plays a key role in the progression of early PCa cancer and the progression from metastatic disease to CRPa.

2.4.3 PROSTATE-SPECFIC ANTIGEN

Prostate-specific antigen (PSA) is a serine protease produced by prostatic tissue and is used as a clinical test for prostate cancer.[192] A glycoprotein, it consists of a 237 amino acid protein appended with a carbohydrate chain.[243] In normal prostate tissue, PSA is secreted into the glandular ducts, where it is highly concentrated. PSA only reaches circulation if there is "leaking backwards" from the duct into the extracellular fluid and into circulatory plasma.[244] In prostate cancer, the structure of the prostate is changed and malformed. As a result, there is an increased amount of PSA directly secreted into the extracellular fluid and, as such, into the plasma.[244]

PSA level has long been used as a clinical test for prostate cancer, however, in recent years there has been less reliance on PSA level as an indicator for prostate disease. [244] The United States Preventive Services Task Force recently ruled that the PSA level should not be used as a disease marker for prostate cancer, as it results in an overdiagnosis of otherwise asymptomatic PCa.[245] A PSA level of between 4-10 ng/mL is reported as "suspicious" and warrants further assessment.[218] More often, the PSA velocity is considered. The velocity is the rate of change of PSA levels, which is most relevant once a tumour reaches the size when it requires vascularisation for continued

growth, approximately 1-3 mm in diameter.[246] From this point, direct blood supply access to the tumour increases the relevance of the PSA test. PSA level is a clinical indication of AR activity[247] not for the presence of prostate cancer.

2.4.4 PROSTATE-SPECIFIC MEMBRANE ANTIGEN

Prostate-specific membrane antigen (PSMA) is a folate hydrolase that is expressed in primary and metastatic prostate cancer.[248] Expression is further upregulated in CRPa and in aggressive high-grade tumours.[249,250] PSMA is not expressed in the vasculature of healthy non-prostatic tissue. The crystal structure for PSMA was solved by Davis *et al*. in 2005, revealing a zinc binding site and a substrate binding arginine patch (Figure 12).[251]

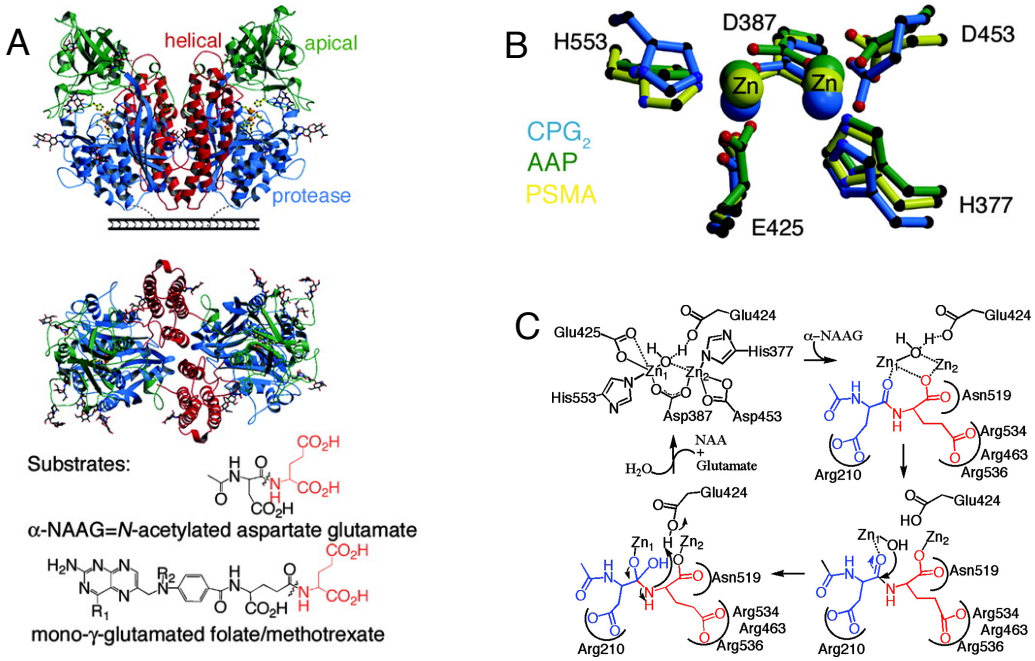
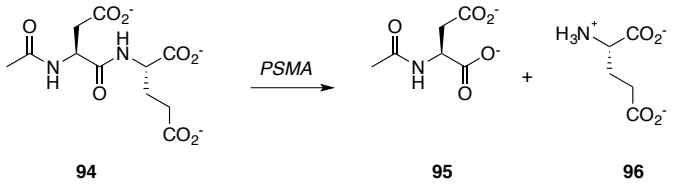


Figure 12: Crystal Structure of PSMA. A.) Ribbon diagrams of side and top view of PSMA with NAAG **94** and methotrexate bound; B.) Overlay of zinc binding site of PSMA with similar enzymes; C.) Proposed role of Zn in a-NAAG cleavage. Adapted from Davis M. I. *et al, Proc. Natl. Acad. Sci.* **2005**, *102*, 5981-5986. Copyright held by The National Academy of Sciences of the United States of America, 2005.

The physiological role of PSMA in the prostate is not known. However, it is known to cleave glutamate from *N*-acetylaspartyl glutamate **94** (NAAG) (Scheme 28).[252,251]



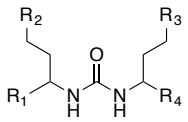
94 **95** **96**

Scheme 28: Hydrolysis of NAAG **94** to acetyl aspartate **95** and glutamate **96**.

PSMA shuttles between the cell interior and cell surface for further internalisation.[253] The combination of these attributes has led to PSMA being identified as a potential target for tumour targeted drug delivery, and as a prognostic biomarker.

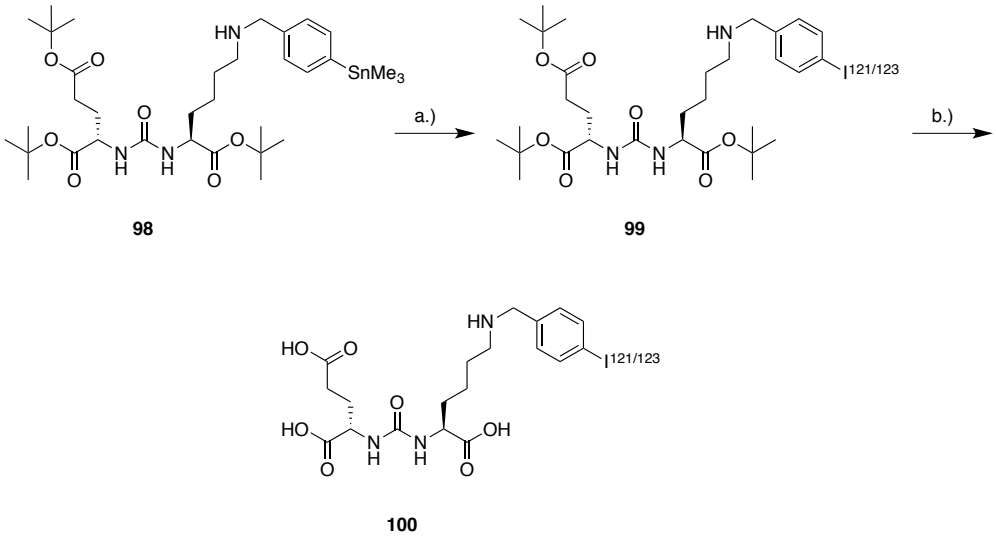
Currently, an indium-111 labelled anti-PSMA antibody is used to detect soft tissue metastasis and disease recurrence, which targets PSMA while internalised.[254] Radiolabelled monoclonal antibodies have also been shown to target cell surface PMSA,[255] but their long-circulating half-life and poor tumour penetration limit their clinical effectiveness.

In light of this, Kozikowski *et al.*[256] developed a series of dipeptide PSMA inhibitors **97**, which, rather than being traditional amide (C-N) linked dipeptides, they are linked by a urea (N-(CO)-N).[256]



97

Maresca *et al.*[257] developed a Glu-Lys conjugate, introducing radiolabelled iodine-123 and iodine-121 isotopes for imaging and therapy respectively (Scheme 29, **100**), by radioiododestannylation (**Scheme 29**).[257] These two molecules demonstrated high affinity for PSMA in LNCaP cells.[257]



98 **99** **100**

Scheme 29: *Reagents and conditions*: a.) [$^{121/123}$I]NaI, H_2SO_4, AcOH, H_2O_2; b.) TFA/CH_2Cl_2, >90% radiolabelled product (over two steps).

2.5 TREATMENT OF PROSTATE CANCER

The treatment strategies for PCa can be divided into five categories, however, not all are available to patients at each stage of the disease. In this section, each treatment option is detailed.

2.5.1 WATCHFUL WAITING

For some patients in the early stages of the disease where the tumour remains isolated in the prostate, there is often no treatment prescribed. A course of "watchful waiting" is more often undertaken. In this instance, the patient is closely monitored by a general practitioner to evaluate the disease progression. This is most often prescribed to elderly patients, or those who are considered high-risk candidates for surgery or radiotherapy.[258]

2.5.2 SURGERY

At present there remains two key surgical options for PCa patients; Radical prostatectomy is a treatment option for patients in Stages I and II of PCa, where the carcinoma remains confined to the prostate. Radical prostatectomy has been reported as a treatment for PCa since 1904,[259] although it is associated with significant morbidity and mortality. In 1947, Millin described the radical retropubic prostatectomy which was undertaken with a single incision.[260] This procedure was the standard of care until the late 1990s and was curative in most men.[261] Modern prostatectomies are carried out laparoscopically, and increasingly with the use of robotics. On average, the 5-year biochemical-free survival rate, that is without increased levels of PSA, is 94%.[262]

Surgery is also an option for androgen ablation therapy. Bilateral orchiectomy, the surgical removal of the testes, has shown to be an effective treatment for stage IV metastatic disease.[263]

2.5.3 RADIOTHERAPY

The treatment of PCa using radiation is offered to patients in Stages II and III of the disease, where the PCa is still confined to the pelvic region. Traditional external beam radiotherapy has been used extensively for the treatment of localised PCa,[264] but in recent years, new radio-therapeutic options are becoming available.[265] For example, conformal radiotherapy uses beams of different shapes to minimize the impact on healthy tissues. The use of 3D MRI and CT imaging allows the tumour to be mapped in three dimensions. Intensity-modulated radiation therapy uses beams of radiation with different applied dosage, to vary the dose delivered to different parts of the tumour.[266]

In addition to external radiotherapy, radiation can be delivered internally in the form of brachytherapy. In permanent brachytherapy radioactive seeds are implanted into the

prostate gland and low dose radiation (2 Gy h^{-1}) is released over a period of weeks. In general, brachytherapy uses isotopes with a long half-life, preferably a few weeks. After this time, the non-radioactive material remains in the patient. Isotopes with short half lives are sometimes used for high-dose (12 Gy h^{-1}), temporary therapy. Following temporary treatment, the radiation source is removed. Iodine-125 ($t_{1/2}$ = 59.6 d),[267] palladium-103 ($t_{1/2}$ = 17 d)[268] and caesium-131 ($t_{1/2}$ = 9.7 d)[269] are used for permanent brachytherapy, and iridium-192 ($t_{1/2}$ = 74 d)[270] is used for temporary brachytherapy.

For advanced PCa with bone metastases, the radiopharmaceutical radium-223 ($t_{1/2}$ =11/4 days) (Alpharadin) is undergoing clinical trials.[271,272] Radium-223 naturally self-localises to bone metastases as a calcium-mimic.[271] Administered monthly for four to six months, Ra^{223} resulted in an increased survival rate of 4.5 months in endocrine resistant tumours.[271]

2.5.4 ANDROGEN ABLATION THERAPY

Prostatectomy and radiotherapy are not treatment options when PCa has metastasised beyond the pelvis. At this point, androgen ablation therapy (AAT) is the primary treatment. As previously indicated in Chapter Two, PCa is an endocrine tumour, requiring testosterone and its metabolite dihydrotestosterone, to continue to grow. AAT's work by suppressing testosterone activity in tumour growth, either by blocking its production or its activity. AAT can be achieved surgically or medically. Bilateral orchiectomy, the surgical removal of the testes, was the "gold standard" in androgen ablation therapy until the 1980's.[273] As the testes are the main source of testosterone production, their removal results in a rapid and permanent reduction in testosterone levels, with a median increase in survival rate of up to 36 months. The advantages of

orchiectomy are its cost compared to long-term medical options, however, potential physical and psychological side-effects led to the development of medical alternatives.

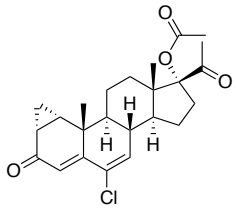
The production of testosterone by Leydig cells in the testes is in response to lutenising hormone (LH). LH secretion is stimulated in response to the secretion of gonadotropin-releasing hormone (GnRH) by the hypothalamus. However, continuous production of GnRH has the opposite effect, it inhibits the release of LH from the pituitary gland, and, as a result, testosterone production from the testes ceases.[274] GnRH agonists are synthetic peptides based on natural GnRH. They act by binding to the GnRH receptor, activating production of LH. However, synthetic modifications result in slow dissociation from the GnRH receptor, resulting in continuous LH production and the subsequent inhibition of testosterone production.[233] GnRH agonists are decapeptides, with synthetic modifications in the tenth and/or sixth position (Table 6).[275]

Agonist	Sequence									
GnRH	pyroGlu	His	Trp	Ser	Tyr	**Gly**	Leu	Arg	Pro	**Gly-NH_2**
Leuprolide	pyroGlu	His	Trp	Ser	Tyr	**D-Leu**	Leu	Arg	Pro	**NHEt**
Buserelin	pyroGlu	His	Trp	Ser	Tyr	**D-Ser(OtBu)**	Leu	Arg	Pro	**NHEt**
Nafarelin	pyroGlu	His	Trp	Ser	Tyr	**D-Naphyl**	Leu	Arg	Pro	**Gly-NH_2**
Histrelin	pyroGlu	His	Trp	Ser	Tyr	**D-His(*N*-Bn)**	Leu	Arg	Pro	**NHEt**
Goselerin	pyroGlu	His	Trp	Ser	Tyr	**D-Ser(OtBu)**	Leu	Arg	Pro	**$NHNHCO_2NH_2$**

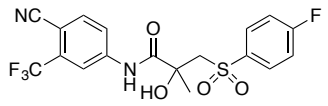
Table 6: GnRH agonists, position 6 and 10 highlighted.

GnRH agonists have the same clinical efficacy as surgery, however, the early stages of GnRH agonist treatment are associated with "tumour flare". The initial surge of testosterone production results in a rapidly growing tumour, which is associated with pain, urinary tract blockages and potential spinal compression.[276] As a result of this, GnRH agonists are often prescribed in conjunction with a second class of androgen ablation therapy; anti-androgens.

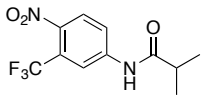
Anti-androgens are androgen receptor antagonists.[277] The binding of anti-androgens halts the cascade of biochemical signals that result in tumour growth. Four key anti-androgens are used clinically, cyprostat **101**, bicalutamide **102**, flutamide **103** and nilutamide **104**.[278]



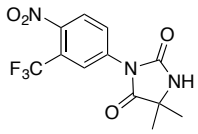
101



102



103



104

Cyprostat **101** is a synthetic derivative of 17-hydroxyprogesterone.[279] Key modifications to the steroid backbone include a C2-C3 cyclopropanation, chlorination at C5, with corresponding C5-C6 oxidation to the alkene, and acetylation of the alcohol at C17. These modifications furnish cyprostat **101** with potent antagonist activity for the androgen receptor the drug shows limited metabolism. Cyprostat **101** also displays inhibitory activity in the biosynthesis of endogenous corticosteroids.[280]

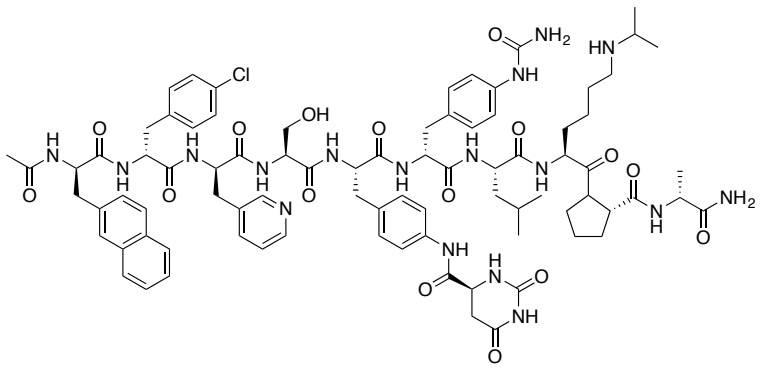
The aryl amides bicalutamide **102**,[281] flutamide **103** [282] and nilutamide **104**[283] act as pure anti-androgens by competitive inhibition of the androgen receptor. bicalutamide **102** also accelerates the degradation of the androgen receptor and has replaced flutamide **103** and nilutamide **104** in most instances.

Key to each structure is the electron-withdrawing aromatic ring, containing a trifluoromethyl and nitro or nitrile group. This is required for efficient binding to AR.[284] The hydroxyl group of bicalutamide **102** (and the active metabolite of flutamide **103**) is responsible for a key interaction with the AR. [285] Anti-androgens are widely used for the

treatment of Stage IV metastatic disease, either as a mono-therapy or in conjunction with GnRH agonists. However, patients with advanced PCa eventually develop resistance to anti-androgens.

In addition to the above described treatments for PCa, two new mechanisms for androgen ablation are reaching the clinic. The first is the use of a GnRH antagonist, the second, CYP17 inhibition.

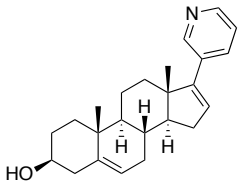
GnRH antagonists bind to the GnRH receptor.[286] However, while GnRH agonists rely on the body's biochemical response to massive LH production, and subsequent desensitisation to its effects, GnRH antagonists competitively and reversibly bind to the GnRH receptor, blocking the release of LH. The biological response also results in suppression of testosterone production. The advantage of GnRH antagonists is that they have an immediate action of lowering testosterone production, without the associated "tumour flare". Currently, only one GnRH antagonist is marketed, degarelix **105**. Degarelix **105** is a synthetic decapeptide containing six non-proteinogenic amino acids.[287]



105

CYP17 inhibition is the most promising recent advance in the treatment of advanced PCa, and CRPa.[288] CYP17 is a cytochrome P450 enzyme involved in the biosynthesis of testosterone from cholesterol.[289] Inhibition of this enzyme prevents the biosynthesis of testosterone in any cell throughout the body, and is not limited to those generally

associated with testosterone production.[288] CYP17 inhibition is most effective in CRPa, where one of the postulated mechanisms for its development is increased testosterone production from previously low contributors, such as the adrenal glands.[290] The CYP17 inhibitor abiraterone **106** is currently marketed for the treatment of CRPa.[291] The development and detailed mechanism of action of abiraterone **106** is discussed extensively in Chapter Four.



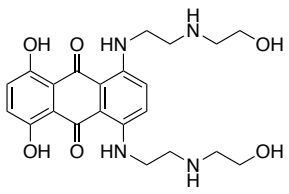
106

Androgen ablation therapy results in a 90-95% decrease of testosterone levels in the blood[292] and up to 90% reduction in intraprosatic levels of DHT, and is effective in up to 80% of patients.[293] This reduction in DHT results in apoptosis of prostate cancer cells, and prostate epithelial progenitor cells. In addition, ablation therapy results in the apoptosis and degeneration of prostatic capillaries, and constriction of larger blood vessels supplying the prostate preceding apoptosis. Tumour vasculature survival is regulated by paracrine growth factor regulation between epithelial stromal cells.[294] As such, while blood vessel growth does not require androgens, their growth is regulated by basic fibroblast growth factor and VEGF, which in turn, is regulated by the AR.[294] Inhibition of AR activity consequentially results in local vasculature apoptosis, compounding the prostate cancer apoptosis.[295]

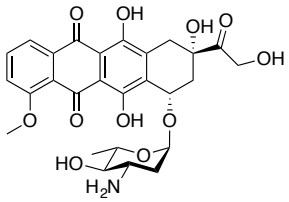
2.5.5 CHEMOTHERAPY

Patients who fail to respond to initial androgen therapy, or who relapse with CRPa have limited treatment options after abiraterone. Cytotoxic chemotherapeutic agents remain

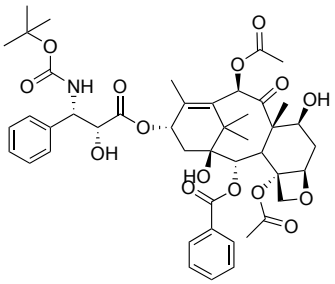
the mainstay of treatment at this stage, with mitoxantrone **107**, docetaxel **108**, epirubicin **109** and estramustine **110** often prescribed.[296,297]



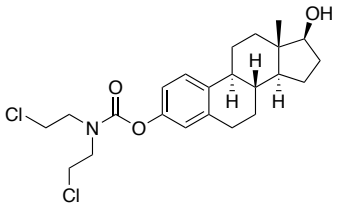
107



109



108



110

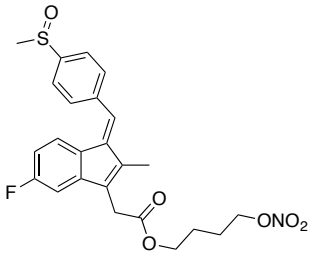
2.6 SUMMARY

Prostate cancer continues to be a challenging disease and developing effective treatments is at the forefront of modern medicine. The absence of definitive biomarkers for disease identification and progression result in difficulties for diagnosis, prognosis and treatment. Key molecular architectures such as the androgen receptor, prostate specific antigen and prostate specific membrane antigen, present themselves as targets for improved treatment of prostate cancer. New pharmaceuticals such as abiraterone and degarelix aim to improve patient outcome when patients develop castration-resistant disease.

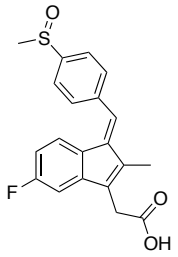
The discovery of new drugs for the treatment of prostate cancer remains important, and the rest of this thesis will discuss the development of novel compounds that release nitric oxide as potential agents for the treatment of prostate cancer.

CHAPTER THREE: NITRIC OXIDE-DONATING ANALOGUES OF SULINDAC

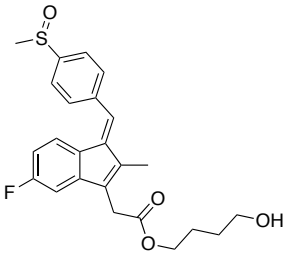
In 2009, it was reported by Stewart *et al.*, that an NO-donating analogue (NCX-1102) **111** of sulindac **112**, a widely used non-steroidal anti-inflammatory drug, was cytotoxic against PC3 cells.[298]



111



112



113

PC3 is a human prostate cancer cell line of hormone insensitive, metastatic disease, isolated from a bone metastasis of a human male.[299] It represents prostate cancer in stage IV, castration resistant disease. Cells at this stage of progression have a high metastatic potential.[300]

It was reported that the NO-donating analogue NCX-1102 **111**, has a pro-apoptotic, cytotoxic, and anti-invasive effect on PC3 cells.[298] In addition, a *des*-nitrato analogue, NCX-112 **113**, where the nitrate ester of NCX-1102 **111** is hydrolysed, was more active that sulindac **112**, but had decreased activity in comparison to NCX-1102 **111**. The cytotoxicity of NCX-1102 **111** was conserved under normoxic and hypoxic conditions.

An investigation into the mechanism of this activity revealed that NCX-1102 **111** was able to reverse the hypoxic response. HIF-1α expression was downregulated under hypoxic conditions upon treatment with NCX-1102 **111**,[298] also under hypoxic conditions, HIF-1α expression was equally expressed when treated with sulindac **112**,

and a DMSO control.[298] NCX-1102 **111** is predicted to function as an NO-donor and as such, the observed increase in cytotoxicity is attributed to 'NO bioactivity'. However, NO in isolation appears ineffective, as the use of sodium nitroprusside, an NO donor, showed no cytotoxicity.[298]

Interestingly, when experiments were conducted with a proteasome inhibitor, the observed reduction in HIF-1α expression was maintained. This suggests that the mechanism of HIF-1α reduction reported by Hagen *et al*.,[52] of oxygen redistribution to allow HIF-1α proteosomal degradation, as a result of NO inhibition of mitochondrial activity,[52] is not at play in this system. In addition to reduced HIF-1α expression, a reduction in Akt phosphorylation was observed and suggested that the PI3K-Akt-mTOR signal transduction pathway is one of the mechanisms operating for the observed activity of NCX-1102 **111**. Previous studies[301–303] have demonstrated a link between this pathway and HIF-1α expression under hypoxic conditions.

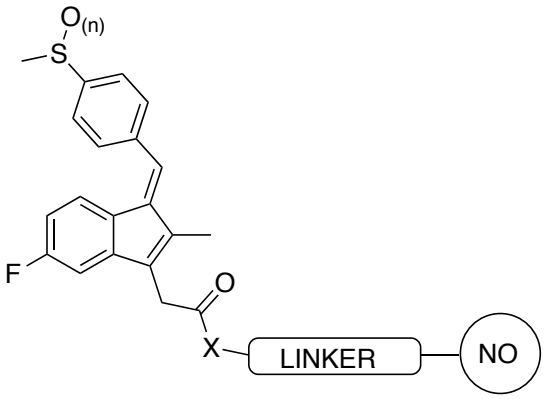
Subsequent observations revealed that in addition to inhibition of the hypoxic response and cytotoxicity, NCX-1102 **111** mediated a radiosensitisation effect on prostate cancer under varying oxygen conditions. The mechanism of radiosensitisation was found to be due to increased DNA double strand breaks, together with fixation of the radiation-induced damage in DNA, which otherwise would be repaired.[304] It was noted that the concentration required to induce radiosensitisation of the PC3 cells were relatively high (IC_{50} values ranging from 10 to 50 μM) suggesting a limited clinical applicability of NCX-1102.[304,305]

Consequently the synthesis of analogues of NCX-1102 **111** that could deliver NO with improved activity for both cytotoxicity and radiosensitisation potential are of interest.

3.1 AIMS

The aim of this work was to design and synthesise a series of NCX-1102 **111** analogues utilising nitrate esters, furoxans and sydnonimines as NO-donating functional groups. These motifs would be appended *via* esters and amides through the sulindac **112** carboxylic acid through a by of linkers. The oxidation state of sulfur will also be changed to assess its affect on activity.

The target molecules have a generic structure based on **114**.

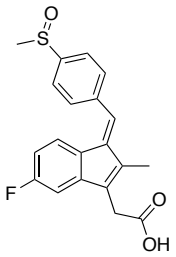


X = NH or O

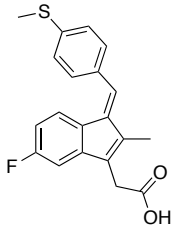
114

3.2 RESULTS AND DISCUSSION

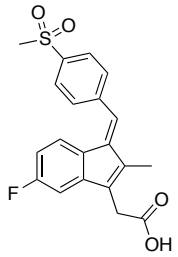
An approach into the re-targeting of sulindac **112** for other therapeutic applications has shown that varying the oxidation state of the sulfoxide **112**, to both the sulfide **115** and sulfone **116** can affect the biological properties of the compounds.[306,307]



112



115

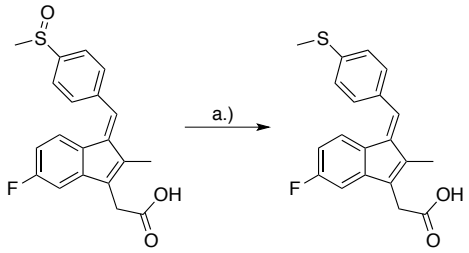


116

To this end, NO-donating analogues of the sulfoxide **112**, sulfide **115** and sulfone **116** were prepared. The sulfide **115** and sulfone **116** oxidation states could be accessed readily and in high yield from the commercially available sulfoxide **112**.[308]

3.2.1 PREPARATION OF SULINDAC SULFIDE AND SULFONE

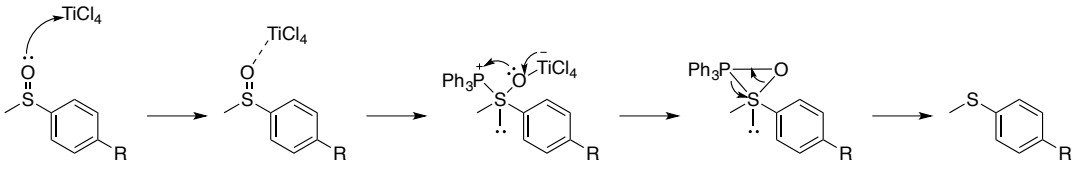
The reduction of sulindac sulfoxide **112** to sulfide **15** has been reported by Folgi *et al.* (Scheme 30).[308] using triphenylphosphine and titanium (IV) chloride, based on a method developed by Kikuchi *et al.*[309]



112 **115**

Scheme 30: *Reagents and conditions*: a.) Ph_3P, $TiCl_4$, THF, r.t., 24 h, 75%.[308]

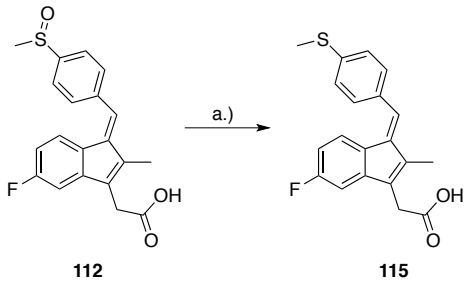
The proposed mechanism of this triphenylphosphine/Lewis acid deoxygenation is illustrated in Scheme 31.[309]



112 **117** **118** **119** **115**

Scheme 31: Proposed mechanism of $PPh_3/TiCl_4$ mediated sulfoxide deoxygenation.[309]

In the event a polymer supported triphenylphosphine was used. Upon consumption of the sulfoxide **112,** the reaction mixture was filtered through a pad of Celite to remove the polymer supported reagent (Scheme 32).



112 **115**

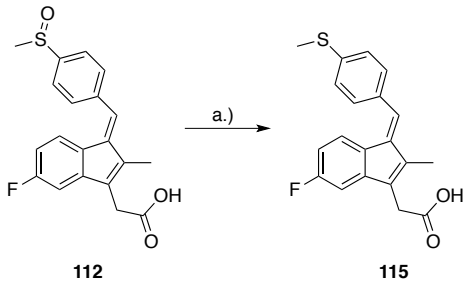
Scheme 32: *Reagents and conditions*: a.) PS-PPh_3, $TiCl_4$, THF, r.t., 24 h, 94%.

This was effective in providing the desired sulfide **115** in 94% yield. ^{31}P NMR spectroscopy confirmed that there was no residual phosphorus residues in the product. While this was effective in providing the first sample of sulfide, it was not suitable for larger quantities due to the high cost of polymer supported triphenylphosphine.[310]

As such standard triphenylphosphine was used, using the procedure reported by Fogli *et al*.[308] However, following work-up, ^{1}H and ^{31}P NMR spectroscopy confirmed the presence of both triphenylphosphine and triphenylphosphine oxide in the samples prepared, which could not be fully removed by trituration or column chromatography.

As such an alternative reduction method was sought. The deoxygenation of sulfinyl groups has been reported to proceed under a variety of conditions,[311] a similar 'low valent' titanium (II), with zinc as the reductant was chosen.[97]

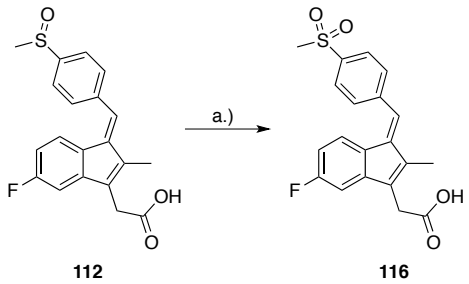
The addition of sulindac sulfoxide **112** to a solution of $TiCl_4$ and zinc powder resulted in complete reduction to the sulfide **115** within 30 minutes and after work up the sulfide **115** was recovered in quantitative yield with no further purification required.



112 **115**

Scheme 33: *Reagents and conditions*: a.) $TiCl_4$, Zn, THF, r.t., 0.5 h, quantitative.

Folgi *et al.* also describe the preparation of sulfone **116**.[308] Oxidation of the sulfoxide using Oxone® under aqueous conditions furnished the desired sulfone, in quantitative yield on a 1 mmol scale also without the need for further purification (Scheme 34).



112 **116**

Scheme 34: *Reagents and conditions*: a.) Oxone®, 1:1 MeOH:H_2O, r.t., 1 h, quantitative.

Scaling this reaction to 2.4 mmol (1 g scale) required column chromatography to purify the product **116**, however this and gave a slightly lower yield (50-70%).

The formation of the oxidation states sulindac sulfoxide **112**, sulfide **115** and sulfone **116** was easily determined by using ^{1}H NMR as shown in Figure 13. The chemical shift of the methyl group attached to the sulfur group shifts depending upon the oxidation state. For the sulfoxide **112,** the $SOCH_3$ is a singlet at 2.81 ppm. The sulfone **116**, is shifted downfield to 3.14 ppm and reduction to the sulfide **15** results in an upfield shift

to 2.55 ppm. Chemical shift changes are were also observed in the ^{13}C NMR signal for the methyl group (sulfide, 15 ppm, sulfoxide 44 ppm and sulfone 46 ppm). Similar changes are also observed in the ^{19}F NMR (Figure 13).

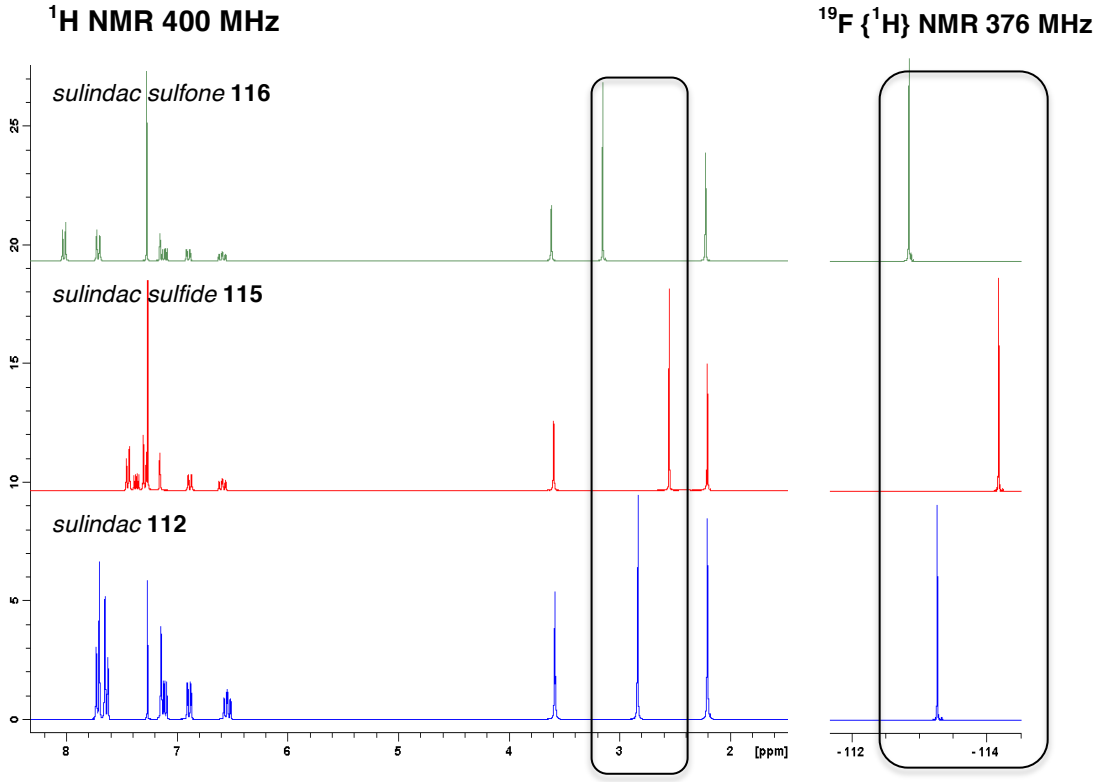


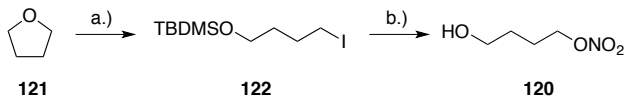
Figure 13: ^{1}H and ^{19}F {^{1}H} NMR ($CDCl_3$) comparison of sulindac sulfoxide **112**, sulfide **115** and sulfone **116**.

3.2.2 NITRATE ESTER-SULINDAC ANALOGUES

The initial task to prepare corresponding 4-nitrooxybutyl and 4-hydroxybutyl esters, analogous of sulfide **115** and sulfone **116**, analogous to NCX-1102 **111** and NCX-112 **113**. In addition, samples of NCX-1102 **111** and NCX-112 **113** were required to be used as positive controls for *in vitro* analysis.

The reported method for preparing the 4-nitrooxybutyl ester of NCX-1102 **111**, is the formation of the 4-bromobutyl ester followed by nitrate displacement. This was replaced with a Steglich esterification[312] using 4-nitrooxybutanol **120**. This limits the

possibility of silver residues contaminating the final product. The required 4-nitrooxybutanol **120** was prepared based a literature procedure (Scheme 35).[313,314]

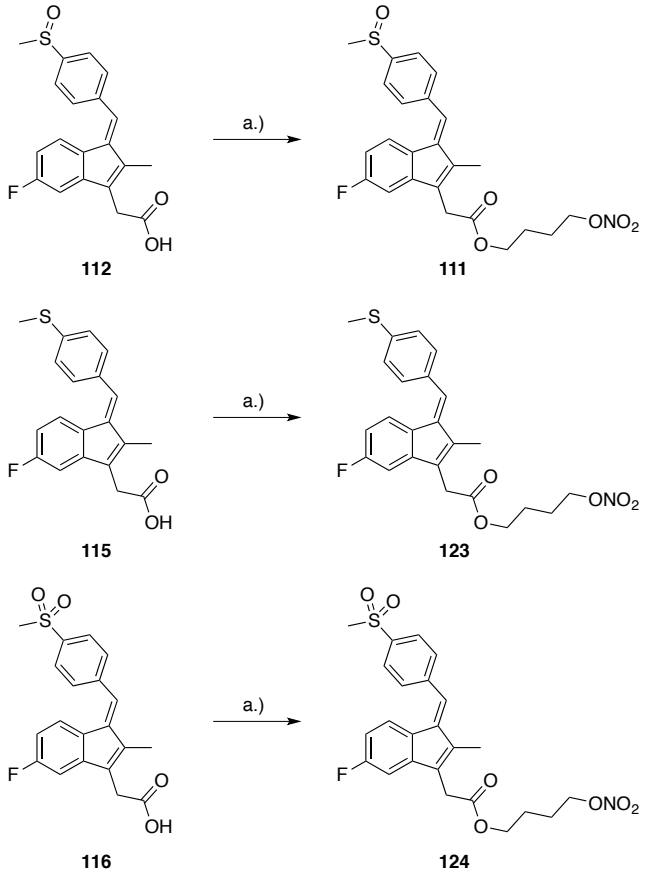


121 **122** **120**

Scheme 35: *Reagents and conditions*: a.) NaI, TBDMSCl, THF, 55 °C, 18 h, quant.; b.) i.) $AgNO_3$, CH_3CN, 1.25 h, ii.) H_2O, 1 h, 27%

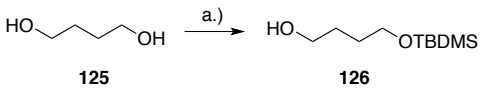
Ring opening of THF with sodium iodide, and trapping of the alkoxide as its TBDMS ether, proceeded in quantitative yield. Immediate treatment of the purified iodide with silver nitrate, followed by acidolysis provided 4-nitrooxybutanol **120** in 27% yield.

Previous work in the group[315] into the preparation of sulindac esters used dicyclohexylcarbodiimide (DCC) as the activating agent in a Steglich esterification. While the esterification was effective the formation of the dicyclohexylurea product was a problem and residues were retained after purification. As such, EDCI.HCl was used as the urea by-product is both water soluble, and easily removed on work-up with an acid wash. In the event the desired esters **111**, **123** and **124** were prepared in good yields from the corresponding carboxylic acids **112**, **115** and **116** (Scheme 36).



Scheme 36: *Reagents and conditions*: a.) **120**, EDCI.HCl, DMAP, CH_2Cl_2, r.t., 3 h, **111** = 91%, **123** = 95%, **124** = 73%.

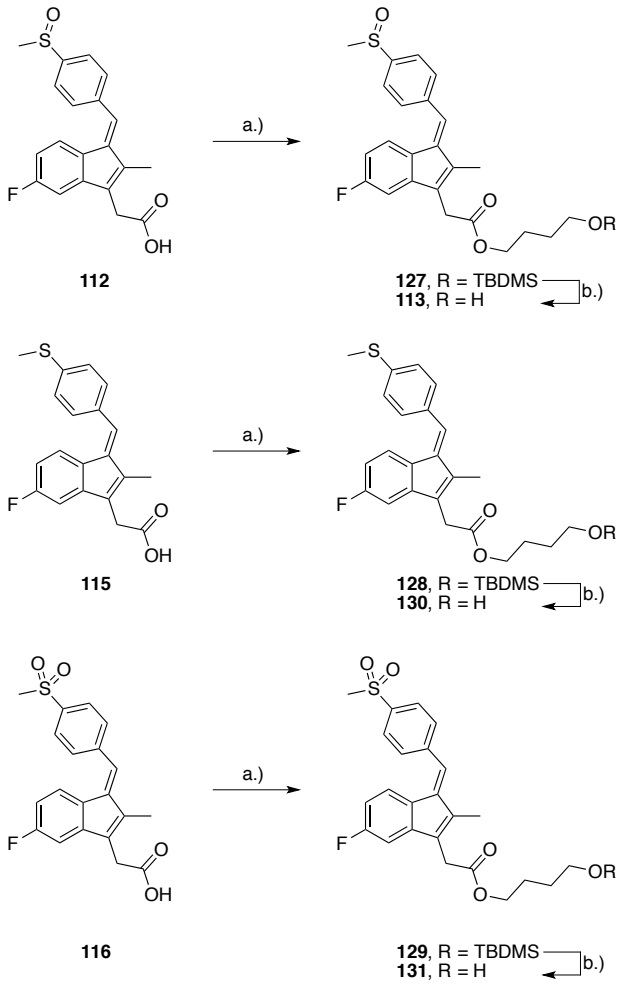
In order to prepare the 4-hydroxybutyl esters, 1,4-butanediol **125** was mono-protected as its TBDMS-ether **126** based on a procedure of Taillier *et al*. This used an excess of 1,4-butanediol **125** to drive mono-protection (Scheme 37).[316]



Scheme 37: *Reagents and conditions*: a.) TBDMSCl, Et_3N, CH_2Cl_2, r.t., 18 h, 68%.

Steglich esterification with carboxylic acids **112**, **115** and **116** furnished the desired TBDMS esters **127-129**, respectively. Deprotection with CSA/MeOH furnished the

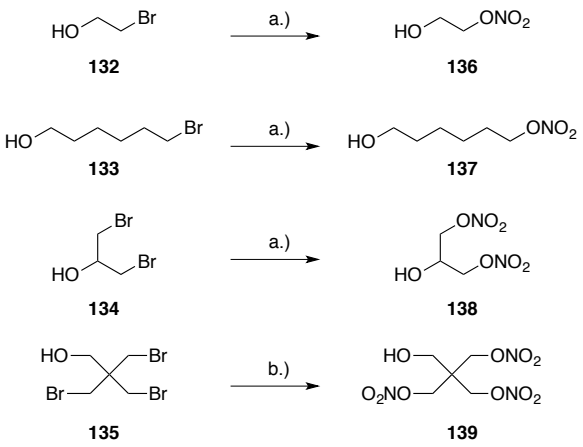
desired 4-hydroxybutyl esters **113**, **130** and **131** in good yields after chromatography (Scheme 38).[317]



Scheme 38: *Reagents and conditions*: a.) **126**, EDCI.HCl, DMAP, CH_2Cl_2, r.t., 3 h, **126** = 85%, **128** = 98%, **129** = 89%; b.) 10-CSA, CH_2Cl_2:MeOH (1:1), r.t., 3 h, **113** = 63%, **130** = 82%, **131** = 75%.

In addition to the 4-nitrooxybutyl esters of sulindac **112**, sulfide **115**, and sulfone **116** a series of alternate nitrate ester alcohols were prepared to investigate the effect of chain length, and nitrate content.

Commercially available bromoalcohols **132-135** were treated with silver nitrate in acetonitrile under reflux, to furnish nitrate esters **136-139** (Scheme 39, Table 7).



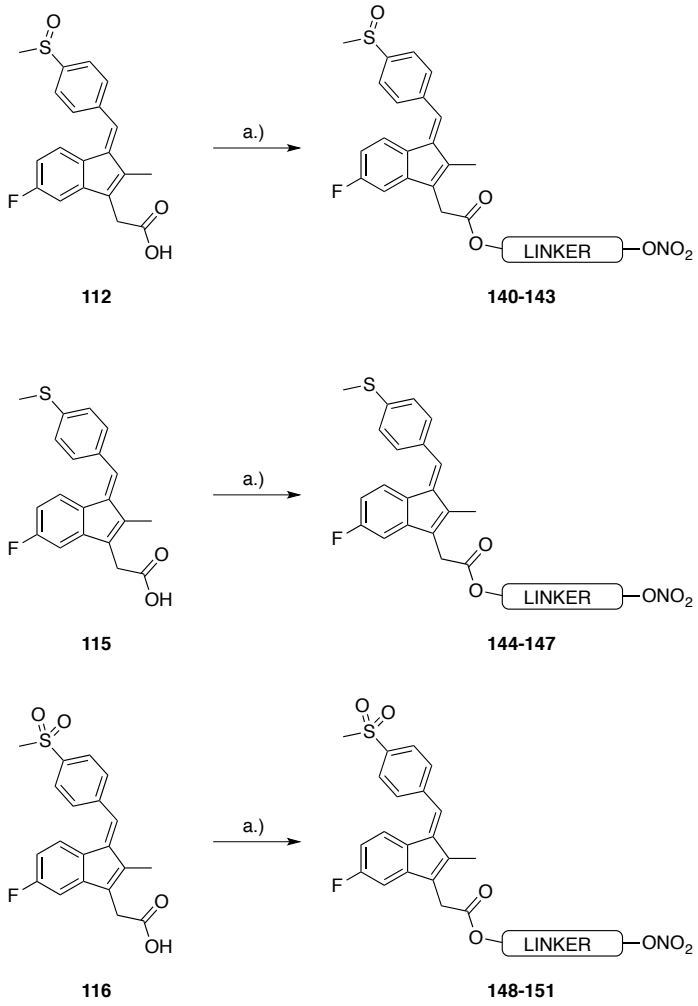
Scheme 39: *Reagents and conditions*: a.) $AgNO_3$, CH_3CN, reflux, 8 h; b.) $AgNO_3$, CH_3CN, r.t., 7 days. See **Table 7** for yields.

Alcohol	Product	Yield (%)
132	**136**	**85**
133	**137**	**90**
134	**138**	**88**
135	**139**	**26**

Table 7: Yields of different nitrate ester alcohols.

Alcohols **136-139** are known[127,318,319] in the literature. Alcohol **139** is known from the de-nitration of PETN **31** with hydrazine hydrate[100] or by hydrolysis of a single nitrate ester with rubidium hydroxide.[320] Given the explosive nature of **31**, neither method was used to prepare alcohol **139**. Rather, pentaerythritol tribromide **135** was treated with a excess of silver nitrate at room temperature over 7 days. After this time, alcohol **139** was isolated in 26% yield after chromatography. While there is limited evidence[100] to suggest that alcohol **139** is explosive, as a precaution it was stored at -20 °C and was not prepared on large scale. With alcohols **136-139**, in hand they were coupled to sulindac **112** and its sulfide **115** and sulfone **116**. Steglich esterification with alcohols **136-139**

furnished the desired esters **140-151** in acceptable to excellent yields (Scheme 40, Table 8).



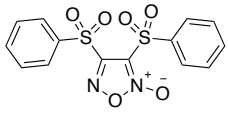
Scheme 40: *Reagents and conditions*: a.) **136**-**139**, EDCI.HCl, DMAP, CH_2Cl_2, r.t., 3 h,

Linker	Product	Yield (%)	Product	Yield (%)	Product	Yield (%)
136	**140**	44	**144**	82	**148**	90
137	**141**	66	**145**	95	**149**	90
138	**142**	80	**146**	85	**150**	92
139	**143**	86	**147**	92	**151**	88

Table 8: Nitrate ester analogues of sulindac.

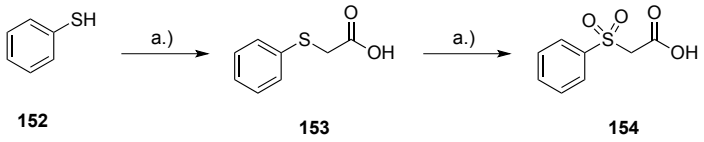
3.2.3 FUROXAN-SULINDAC ANALOGUES

It was envisioned that the furoxan heterocycle could be linked to sulindac **112** by synthesising a series of furoxan alcohols for preparation of sulindac esters. The most widely used furoxan in the literature[321] is *bis*(phenylsulfonyl)furoxan **53** and this has shown NO release properties in *in vitro* and *in vivo* models, so it was chosen as the furoxan motif used for this research.



53

Bis(phenylsulfonyl)furoxan can undergo selective substitution at the 4-position with alcohols and diols.[321] It was prepared in three steps following a literature procedure.[152,322] Alkylation of thiophenol **152** with chloroacetic acid furnished (phenylthio)acetic acid **153** in 97% yield.[152] This was then oxidised using Oxone® in aqueous methanol to provide sulfone **154** (Scheme 41).[322]



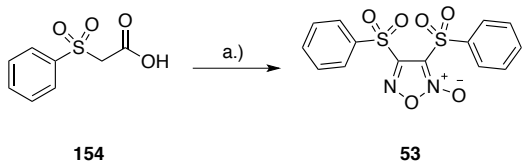
152 **153** **154**

Scheme 41: *Reagents and conditions*: a) Chloroacetic acid, NaOH, Na_2CO_3, 95% aq. EtOH, r.t., 18 h, 97%; b) Oxone®, 1:1 MeOH:H_2O, r.t., 1 h, 95%.

The oxidation state was confirmed to be the sulfone, rather than the sulfoxide by comparison to ^{1}H NMR data in the literature and by mass spectrometry.[323] Oxidation of thioether **153** was conducted on a 1 g scale to provide sulfone **154** without the need for further purification. On scales larger than 1 g, the product required recrystallisation

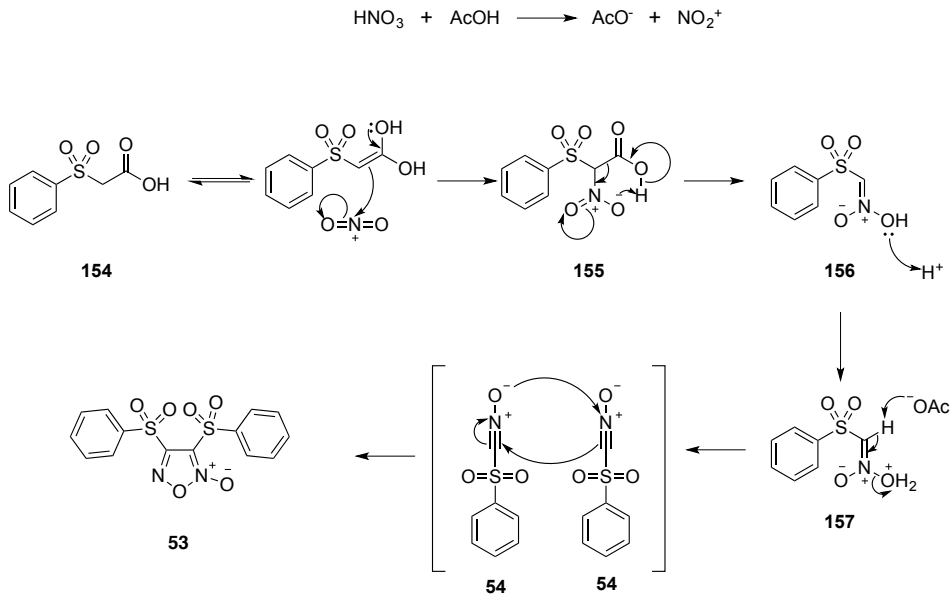
which consequently affected the overall yield. The average yield for the oxidation on a 1 g scale was ~95%.

With the furoxan precursor in hand, cyclisation to *bis*(phenylsulfonyl)furoxan **53** was undertaken. As such, heating a nitrating mixture of fuming HNO_3 and acetic acid furnished the desired furoxan in 50% yield.[152]



Scheme 42: *Reagents and conditions*: a.) fuming HNO_3, AcOH, reflux, 1.5 h, 50%.

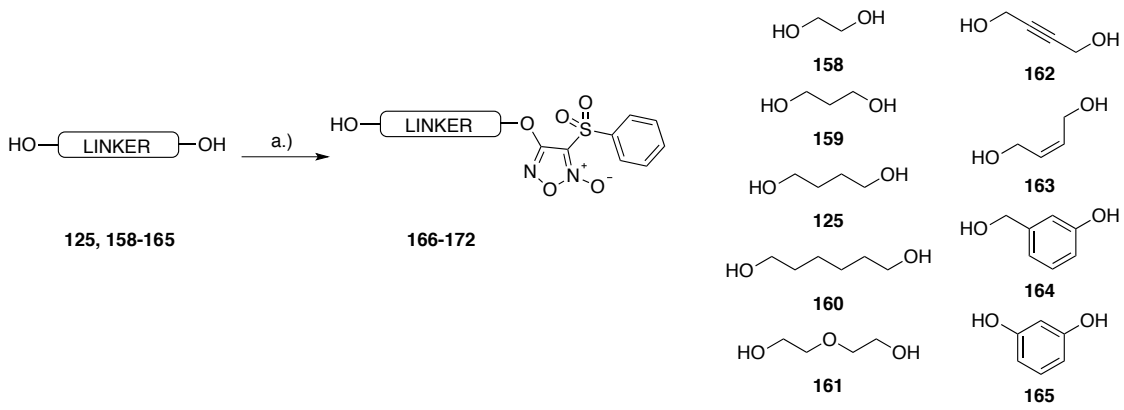
A putative mechanism for the reaction involves an α-nitration to give nitroacid **154**, followed by decarboxylation to the *aci*-nitrosulfone **156**. Loss of water from **157** generates the nitrile oxide **54**, and this species this dimerises by a [3+2] dipolar cycloaddition to the furoxan **53** (Scheme 43). The reaction generates large quantities of deep red "nitrous" fumes for the duration of the reaction.



Scheme 43: Mechanism for the formation of *bis*(phenylsulfonyl)furoxan **53**.

Quenching the reaction mixture into ice-water, precipitated the product as a pale off-white amorphous solid and recrystallisation from isopropanol provided the desired furoxan **53** as white needles. The average yield for this transformation was 50% on a 25 g scale. The literature suggest that reaction times longer than 2 hours result in electrophilic nitration on one of the phenyl rings[321] so this was avoided.

Bis(phenylsulfonyl)furoxan **53** could then be selectively substituted in the 4-position with diols. In total nine diols were chosen link the heterocycles with sulindac. In the event, seven diols generated the desired furoxan alcohols **166-172** in 33-87% yields (Scheme 44, Table 9). Diols **158** and **161** did not give the desired product.

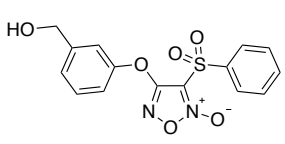


Scheme 44: *Reagents and conditions*: a.) Diol **158-165**, 50% *w/w* aq. NaOH, THF, r.t., 1 h.

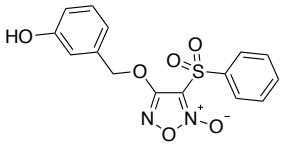
Linker	Product	Yield (%)
159	**166**	67
125	**167**	50
160	**168**	39
162	**169**	82
163	**170**	33
164	**171**	33
165	**172**	54

Table 9: Synthesis of furoxan linkers.

Furoxans **166-169** are reported in the literature,[152] but were fully characterised in this study. Alkene furoxan **170** is reported as a mixture of isomers.[152] The two aromatic linker units **171** and **172** could be prepared in good yield and are the first examples of aromatic linked furoxans. Purification of **172** was only partly successful, however, trituration with chloroform precipitated the starting diol, which could be removed by filtration. 3-Hydroxybenzyl alcohol **164** has two possible alcohol moieties which could potentially give furoxans **171** and **173**. However, only a single phenol adduct **171** was observed.



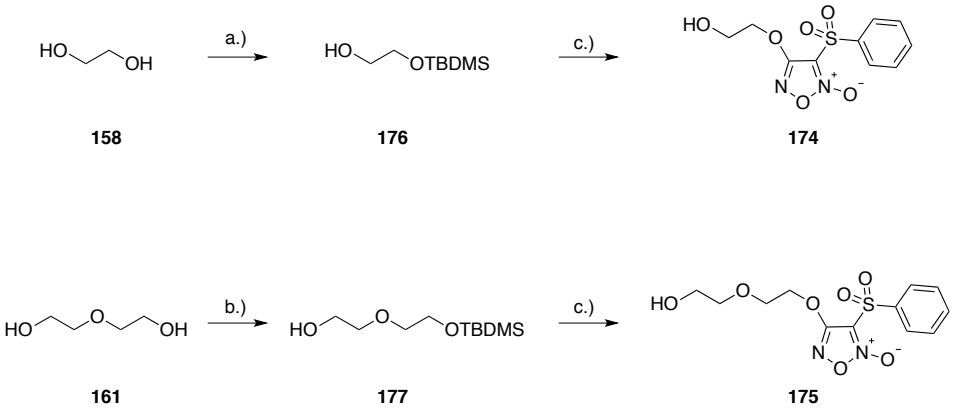
171



173

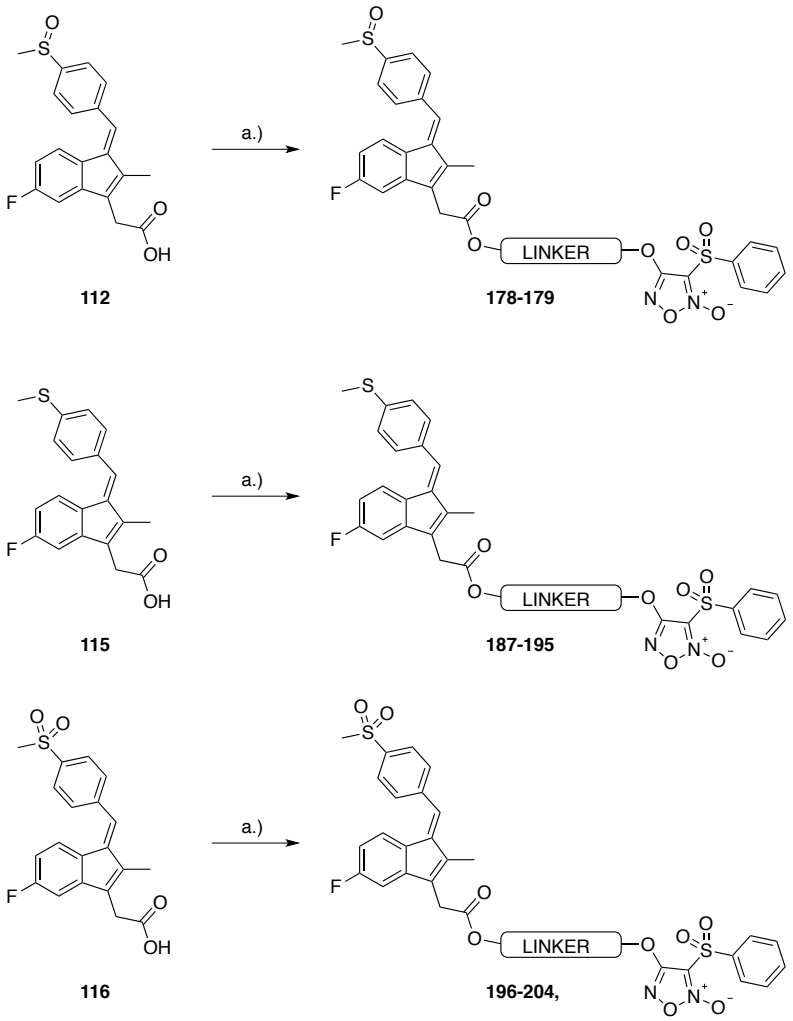
Two diols, **158** and **161,** did not provide the desired furoxan as expected. No product was observed with ethylene glycol **158**. (2-Ethyoxy)ethoxyethanol **161** furnished a mixture of products with the same mass, presumed to be 3- and 4- position isomers.

Preparation of the furoxans **174** and **175**, from diols **158** and **161**, required selective mono-silyl protection with a *tert*-butyldimethylsilyl group to give **176** and **177**. [324,325] This was achieved using an excess of diol relative to *tert*-butyldimethylsilyl chloride (**Scheme 45**). Homologation of the protected diols **176** and **177** with *bis*(phenylsulfonyl)furoxan **53** furnished the desired products. The TBDMS ether was then deprotected by methanolysis (Scheme 45).



Scheme 45: *Reagents and conditions*: a.) TBDMSCl, Et_3N, DMAP, CH_2Cl_2, 0 °C to r.t., 16 h, 74%. b.) TBDMSCl, imidazole, CH_2Cl_2, 0 °C to r.t., 16 h, 50%; c.) i. Diol **176** or **177**, 50% *w/w* aq. NaOH, THF, r.t., 1 h; ii. 10-CSA, MeOH, 0.5 h, r.t., **174** = 60%, **175** = 46%.

With the furoxan alcohols in hand, the final step in the synthesis required the conjugation to the various oxidation states of sulindac **112**, **115** and **116**, respectively (Scheme 46).



Scheme 46: *Reagents and conditions*: **a.) 166-172, 174, 175**, EDCI.HCl, DMAP, CH_2Cl_2, r.t., 2 h.

Linker	Product	Yield (%)	Product	Yield (%)	Product	Yield (%)
174	**178**	89	**187**	46	**196**	25
166	**179**	85	**188**	46	**197**	77
167	**180**	57	**189**	32	**198**	26
168	**181**	83	**190**	80	**199**	71
175	**182**	34	**191**	41	**200**	62
170	**183**	93	**192**	55	**201**	53
169	**184**	68	**193**	56	**202**	32
171	**185**	68	**194**	65	**203**	66
172	**186**	54	**195**	49	**204**	50

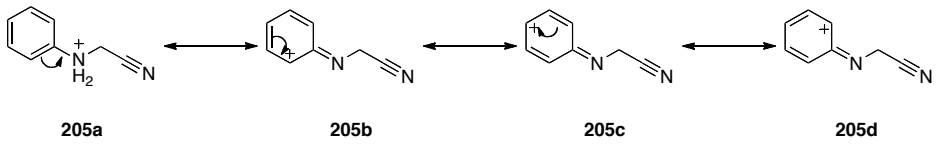
Table 10: Summary of sulindac-furoxan ester yields.

In total, 27 sulindac-furoxan hybrids **178-204** were prepared for biological evaluation, using the 9 furoxan linkers, and the 3 oxidation states of sulindac. The hybrid esters were isolated as yellow solids or gums.

3.2.4 SYDNONIMINE-SULINDAC ANALOGUES

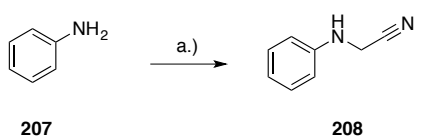
Two key synthetic targets were identified for the conjugation of a sydnonimine to sulindac **112**. These were direct sulindac amides, and attached with a linker between the units.

The sydnonimine chosen was substituted with a phenyl group in the 3-position. This was selected, as it is structurally analogous to the phenyl in the furoxans. The phenyl group should stabilise the cation **205a-d** formed from NO release in a similar manner to a dialkylamino group (Scheme 47).



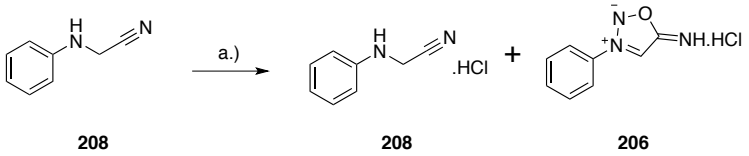
Scheme 47: Stabilisation of phenyl aminoacetonitrile cation **105** by resonance.

The desired sydnonimine **206** was prepared from aniline **207** in two steps (**Scheme 48**). Aniline was alkylated with bromoacetonitrile based on a literature procedure,[326] providing the desired phenylaminoacetonitrile **208** in 87% yield as transparent crystals.



Scheme 48: *Reagents and conditions*: a.) $BrCH_2CN$, K_2CO_3, NaI, CH_3CN, reflux, 18 h, 87%

Phenylaminoacetonitrile **208** was nitrosated with isopentyl nitrite (1.5 equiv., 2 h) in diethyl ether following the procedure reported of Beal and Turnbull.[94] Dry HCl gas was then bubbled through the solution to promote ring closure. This generated a pale pink solid (Scheme 49).

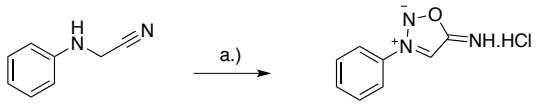


208 **208** **206**

Scheme 49: *Reagents and conditions*: a.) i. Isopentyl nitrite (1.25 equiv.), Et_2O, 2 h, ii. dry HCl (g), 15 min.

^{1}H NMR analysis of the product indicated this to be a 1:1 mixture of the desired 3-phenylsydnonimine HCl salt **206** and phenylaminoacetonitrile HCl salt **208**. This mixture of products could clearly arise be due to the breakdown of the intermediate *N*-nitrosoamine, before HCl addition, as they have been reported to be light sensitive.[80]

The Beal and Turnbull procedure was modified increasing the equivalents of isopentyl nitrite (3 equiv.) and time (16 h) in the absence of light. This protocol provided the desired 3-phenylsydnonimine hydrochloride in 94% yield (Scheme 50).

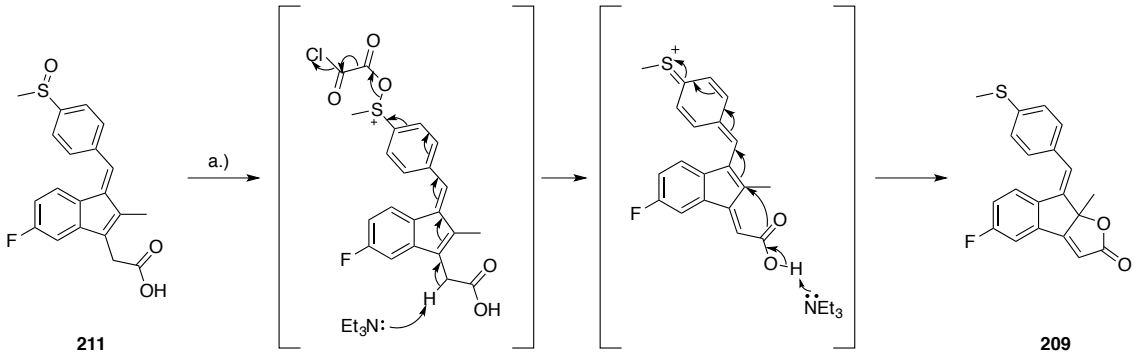


208 **206**

Scheme 50: *Reagents and conditions*: a.) i.) Isopentyl nitrite (3.0 equiv), Et_2O, 16 h, ii) dry HCl (g), 15 min, 94%.

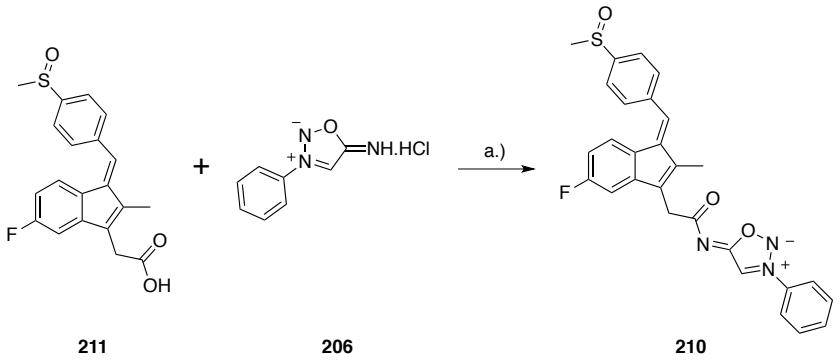
The acylation onto sulindac **112** was now considered. While acylation of sydnonimines onto acyl chlorides has precidence in the literature,[165] there are no reported examples of preparing the acyl chloride of sulindac. Attempts by Halder *et al.*[327] to prepare this acyl

chloride using Vilsmeier conditions (oxalyl chloride/DMF) resulted in formation of sulindac sulfide lactone **209** as the sole product (Scheme 51). This was rationalised as a long-range vinylogous Pummerer rearrangement.[327] In the light of this, a carbodiimide coupling strategy was chosen.



Scheme 51: *Reagents and conditions*: a.) $(COCl)_2$, Et_3N, EtOH, CH_2Cl_2, r.t. 66%.[327]

Under the previously optimised conditions for esterification (EDCI/DMAP/CH_2Cl_2) the reaction was slow and after extended reaction time (18 h) mainly starting material remained (Table 11).



Scheme 52: *Reagents and conditions*: see Table **11**.

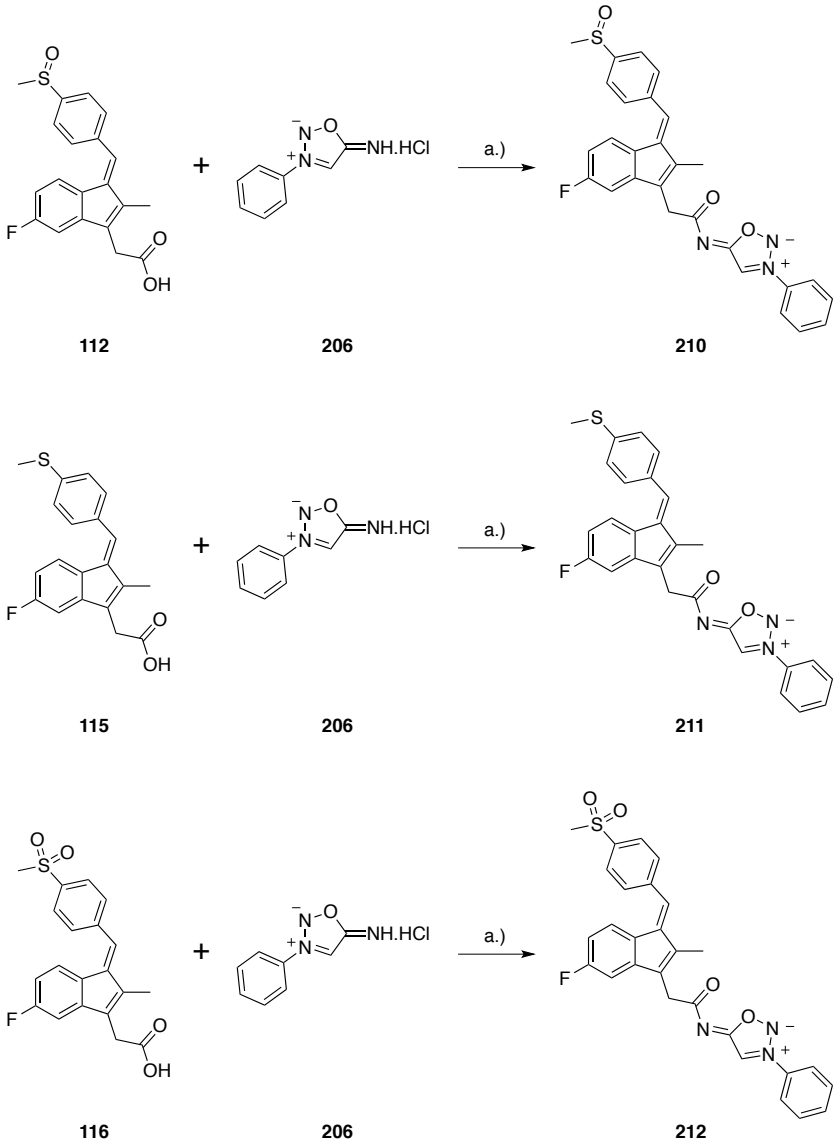
entry	Amine eq.	Solvent	Additive	T/ °C	t/ h	Yield (%)
1	1.1	CH_2Cl_2	EDCI.HCl	r.t.	2	No reaction
2	1.1	CH_2Cl_2	EDCI.HCl	reflux	2	No reaction
3	1.1	CH_2Cl_2	EDCI.HCl + Et_3N	r.t.	2	No reaction
4	1.1	DMF	EDCI.HCl	r.t.	2	35%[a]
5	1.1	CH_2Cl_2:DMF (10:1)	EDCI.HCl	r.t.	2	50%[a]
6	1.1	CH_2Cl_2:CH_3CN (8:2)	EDCI.HCl	r.t.	2	88%

a.) contaminated with DMF

Table 11: Optimisation of conditions for amide **210** formation.

3-Phenylsydnonimine hydrochloride **206** was poorly soluble in dichloromethane and this presented a problem. Addition of 1 equivalent of triethylamine did not improve the reaction. Sydnonimines are poor nucleophiles and are unstable as the free base.[328] It is presumed that the rate of breakdown of the sydnonimine was greater than the rate of acylation. By changing the sovlent to DMF, the solubility improved and the reaction proceeded to give the desired product **210** in *ca*. 35% yield, however removal of DMF on work up proved problematic.

A co-solvent mixture of 10:1 CH_2Cl_2/DMF increased the yield to 50%. In this instance, the solubilty problem was solved, but again, residual DMF contaminated the product after chromatography. Switching from DMF to acetonitrile as a co-solvent (CH_2Cl_2:CH_3CN, 8:2) improved the yield (88%) significantly. With these optimised conditions, the sydnonimine amides of sulindac sulfide **115** and sulindac sulfone **116** were also prepared in 70% and 75% yields respectively (Scheme 53).

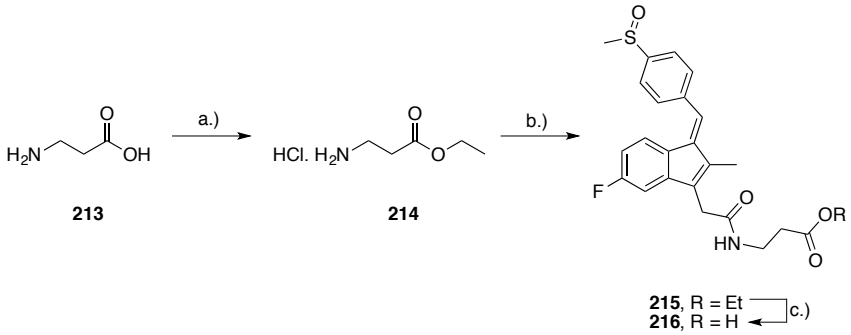


Scheme 53: *Reagents and conditions*: a.) EDCI.HCl, DMAP, CH_2Cl_2/CH_3CN (8:2), 2 h, **210** = 88%, **211** = 70%, **212** = 75%.

The preparation of these amides with sulindac represents the first use of a carbodiimide to prepare sydnonimine amides; upto this point the only reported amides were prepared using acid chlorides. A strategy was now devised for the preparation of sulindac sydnonimine conjugates using a linker.

The first linker considered was the amino acid, β-alanine **213**. It was envisaged that this 3-carbon unit could be attached to the sydnonimine **206** as an amide. β-Alanine **213** was

protected as an ethyl ester **214** and purified by recrystallisation from ethanol and diethyl ether (Scheme 54).[329]

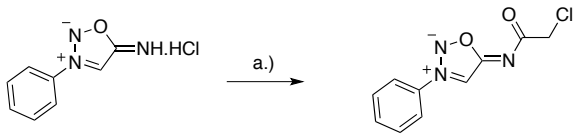


Scheme 54: *Reagents and conditions*: a.) $SOCl_2$, EtOH, reflux, 4 h, quant., b.) DCC, Et_3N, CH_2Cl_2, reflux, 4 h, 91%; c.) LiOH, THF:H_2O (4:1), 16 h, r.t., 76%.

Coupling to sulindac **112** was achieved using DCC with the addition of triethylamine and DMAP to give amide **215** (Scheme 54). Deprotection with aqueous base furnished the homologated carboxylic acid **216** (Scheme 54).

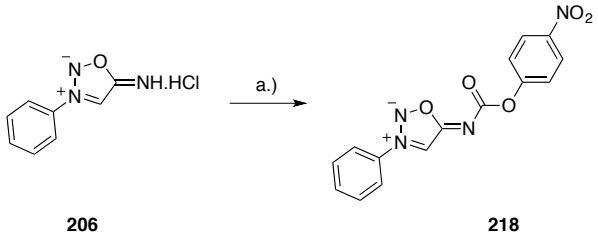
Attempts to form a sydnonimine amide of carboxylic acid **216** using the previous EDCI conditions (EDCI, DMAP, CH_2Cl_2/CH_3CN) were unsuccesful. From here, a series of conditions using a variety of coupling reagents (EDCI.HCl, T3P, HATU, and PyBoP) were screened. However, these did not furnish the desired amide. Attempts to make the acyl chloride using Vilsmeier conditions (oxalyl chloride/DMF) or the Ghosez reagent were also unsuccessful. With nowhere reasonable to go, the β-alanine homologation was abandoned.

Given the known reactivity of sydnonimines an alternative strategy was implemented. This involved acylating the sydnonimine with an acyl chloride to generate an amide, containing a functionality that can be used to create a linker. Thus, treatment of 3-phenylsydnonimine **206** with 2-bromoacetyl chloride and an excess of triethylamine (2.6 equivalents), furnished an acylated species in 66% yield. However the product was not the desired α-bromoamide but the α-chloroamide **217** (Scheme 55).



Scheme 55: *Reagents and conditions*: a.) Bromoacetyl chloride, Et_3N, CH_2Cl_2, -20 °C to r.t., 14 h, 66%.

This unexpected halogen exchange is clearly due to an *in situ* Finkelstein reaction with free chloride ion. In the event, this chloride could not be displaced by suitable amine nucleophiles. As a result, an alternative acyl chloride was explored, containing a similar bifunctionality. 4-Nitrophenyl chloroformate[178,180] was reacted 3-phenylsydnonimine hydrochloride **206** and this generated the 4-nitrophenyl carbamate **218** as a white solid (64% yield) (Scheme 56), which was remarkably stable at room temperature.



Scheme 56: *Reagents and conditions*: a.) 4-Nitrophenyl chloroformate, Et_3N, CH_2Cl_2, -20 °C to r.t., 14 h, 64%.

A number of amides were prepared from the acids **112**, **115** and **116** using ethanolamine **219** and ethylenediamine **220** to link with **218**. The use of both a carbamate- and urea-linked sydnonimines allows comparisons to be made regarding NO release from these structures. Ureas are more hydrolytically stable than carbamates, and as such a concomitant hydrolysis and loss of CO_2 is more likely to occur in the carbamate. Pre-formation of the activated imidazolide with carbonyldiimidazole (CDI), followed by

addition of an excess of ethanolamine **219** or ethylenediamine **220** furnished the desired amides **221-226** (Scheme 57). The yields for each amide are summarised in Table 12.

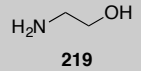
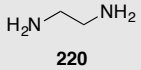
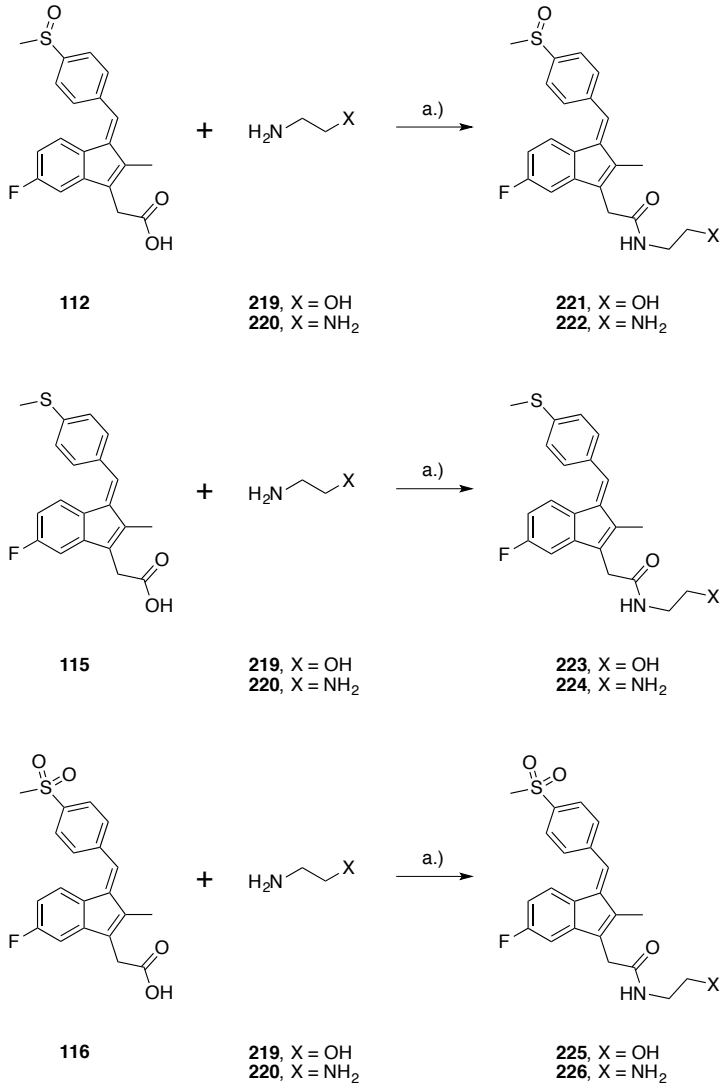
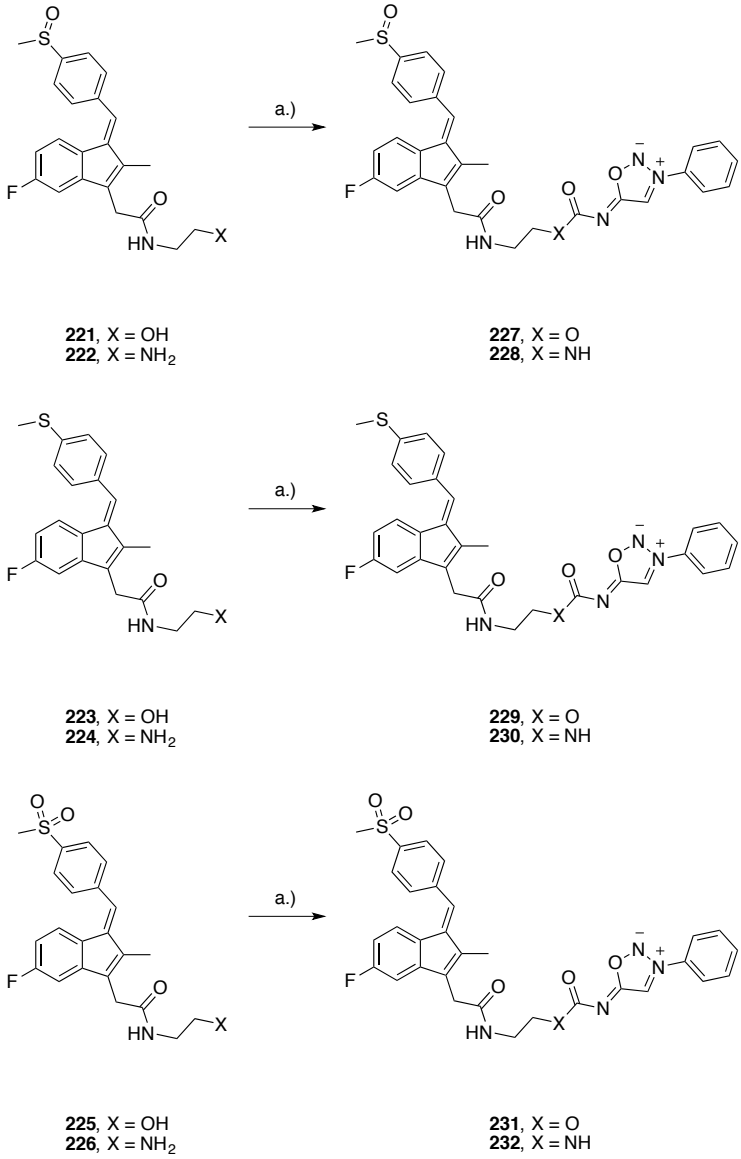
Amine	Product	Yield %	Product	Yield %	Product	Yield %
 **219**	**221**	88	**223**	85	**225**	90
 **220**	**222**	81	**224**	89	**226**	83

Table 12: Summary of yields for the prepared sulindac amides **221-226**.



Scheme 57: *Reagents and conditions*: a.) 1.) CDI, CH_2Cl_2, r.t., 3 h; 2.) **219** or **220**, r.t., 14 h.

Condensation of alcohols **221**, **223** and **225** or amines **222, 224** and **226** with carbamate **217** furnished the requisite carbamates **227**, **229** and **231** and ureas **228**, **230** and **232** (Scheme 58).



Scheme 58: *Reagents and conditions*: a.) **218**, CH_3CN, reflux, 16 h, 17-90%,

Product	Yield %	Product	Yield	Product	Yield
227	17	**229**	56	**231**	49
228	86	**230**	86	**232**	90

Table 13: Summary of the yields of sulindac carbamates and ureas **227-232**.

With the nine sydnonimines in hand, the potential NO-donating analogues of sulindac were subjected to cytotoxicity assays.

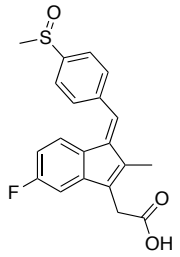
3.3 CYTOTOXICITY RESULTS

The nitrate ester, furoxan, and sydnonimine analogues of sulindac were assayed at the University of Edinburgh for cytotoxicity against PC3 cells using a crystal violet assay. Crystal violet (CV) is a triphenylmethane dye which binds to DNA and is an indicator of cell proliferation. The results are presented as a percentage of cells remaining after treatment with a sulindac analogue. The bound CV gives a strong purple colour that can be examined spectrophotometrically at an optical density of 570 nm. The cell growth percentage is calculated using the following equation:

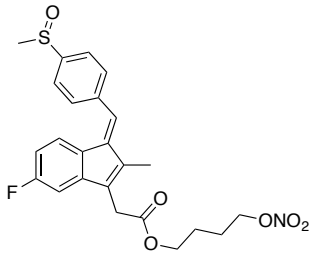
$$\%\ Cells\ remaining = \left(\frac{OD_{570}\ of\ test\ compound}{OD_{570}\ of\ DMSO\ control}\right) \times 100$$

The compounds were screened against PC3 cells under normoxic conditions and at a concentration of 50 μM of the sulindac analogue. The results are summarised in Tables 14-17. Data for compounds which showed statistically significant reduction in cell population are highlighted in bold. Cytotoxicity assays and statistical analyses were conducted by Mr James Black at the University of Edinburgh.

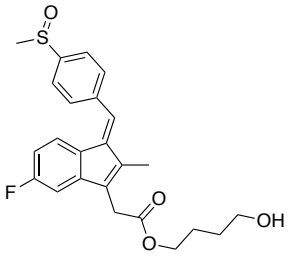
Reference compounds of sulindac **112** its sulfide **115** and sulfone **116** were assayed along with their corresponding 4-nitrooxybutyl and 4-hydroxybutyl esters, **111**, **123-124** and **113**, **130-131**, respectively (Table 14).



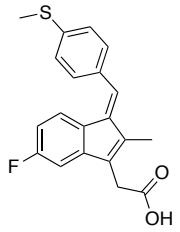
112



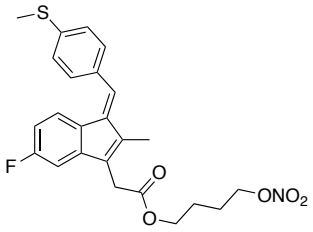
111



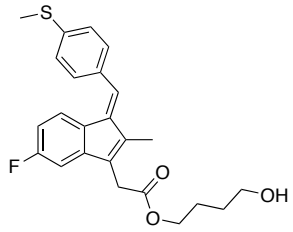
113



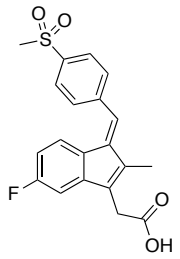
115



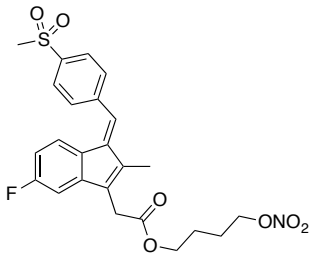
123



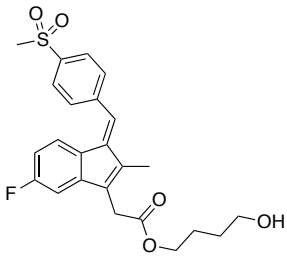
130



116



124



131

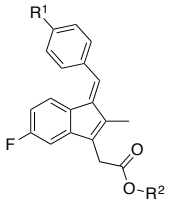
	% Cells alive at time point after treatment at 50 μM[b]		
Entry	24 h	48 h	72 h
DMSO 0.05%[a]	99.1 ± 9.9	100.9 ± 4.9	95.7 ± 7.7
NCX-1102[a]	58.8 ± 8.2	35.5 ± 5.3	24.5 ± 3.9
112	87.3 ± 5.6	101.0 ± 2.4	89.3 ± 7.4
115	99.2 ± 2.9	93.7 ± 5.8	105.4 ± 3.0
116	87.8 ± 2.5	91.0 ± 3.5	83.5 ± 4.9
111	**72.2 ± 9.2**	**43.9 ± 2.9**	**41.0 ± 4.0**
123	**49.4 ± 2.3**	**28.4 ± 2.5**	**17.1 ± 3.6**
124	**68.7 ± 4.1**	**52.9 ± 0.7**	**54.7 ± 3.5**
113	96.6 ± 3.4	76.9 ± 3.9	96.5 ± 3.6
130	98.2 ± 5.8	80.5 ± 6.9	84.0 ± 5.1
131	87.4 ± 7.0	81.5 ± 5.0	86.6 ± 5.3

[a.] Mean of all CV experiments conducted, [b.] Mean of triplicate repeat.

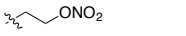
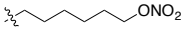
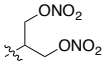
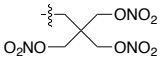
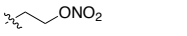
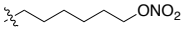
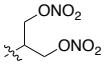
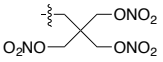
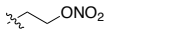
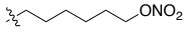
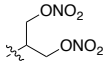
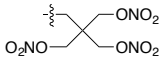
Table 14: Summary of CV cytotoxicity assays of the control compounds.

Sulindac **112** had no cytotoxic effect on PC3 cells, in agreement with the previously reported data by Stewart *et al*.[298] This is also the case for the corresponding sulfide **115** and sulfone **116**. In addition, the 4-hydroxybutyl esters **113, 130-131** did not have any significant cytotoxicity, however, the corresponding 4-nitrooxybutyl esters **111, 123-124** all showed a statistically significant cytotoxic effect, sulindac sulfide 4-nitrooxybutyl ester **123** showed an improved activity over NCX-1102 **111**. Synthetically prepared **111** and NCX-1102 **111** provided by the company NiCOX had different profiles *in vitro*. They were both cytotoxic, but NCX-1102 **111** had in improved activity in the CV assay. The reason for this is unknown.

Table 15 summarises the nitrate ester analogues **140-151**.



140-143: $R^1 = S(O)CH_3$
144-147: $R^1 = SCH_3$
148-151: $R^1 = SO_2CH_3$

140: R = $S(O)CH_3$, R^1 = 
141: R = $S(O)CH_3$, R^1 = 
142: R = $S(O)CH_3$, R^1 = 
143: R = $S(O)CH_3$, R^1 = 
144: R = SCH_3, R^1 = 
145: R = SCH_3, R^1 = 
146: R = SCH_3, R^1 = 
147: R = SCH_3, R^1 = 
148: R = SO_2CH_3, R^1 = 
149: R = SO_2CH_3, R^1= 
150: R = SO_2CH_3, R^1 = 
151: R = SO_2CH_3, R^1 = 

	% Cells alive at time point after treatment at 50 μM[b]		
Entry	24 h	48 h	72 h
DMSO 0.05%[a]	99.1 ± 9.9	100.9 ± 4.9	95.7 ± 7.7
NCX-1102[a]	58.8 ± 8.2	35.5 ± 5.3	24.5 ± 3.9
140	**73.1 ± 24.3**	**54.9 ± 6.8**	**35.5 ± 4.2**
144	95.6 ± 0.8	120.0 ± 13.6	91.2 ± 5.6
148	84.4 ± 16.3	96.2 ± 14.4	84.4 ± 2.7
141	105.0 ± 9.1	72.7 ± 9.3	88.1 ± 22.9
145	92.4 ± 5.1	85.1 ± 8.7	103.7 ± 5.4
149	93.3 ± 17.2	59.0 ± 4.7	64.0 ± 8.0
142	**46.5 ± 2.6**	**44.9 ± 10.3**	**30.2 ± 4.5**
146	91.2 ± 4.6	92.3 ± 1.5	93.6 ± 8.5
150	88.0 ± 11.9	89.0 ± 9.0	89.4 ± 1.2
143	86.0 ± 5.3	79.9 ± 6.4	89.6 ± 6.3
137	88.3 ± 3.6	101.2 ± 6.9	110.4 ± 1.3
151	100.6 ± 8.0	84.5 ± 10.9	95.3 ± 10.0

[a.] Mean of all CV experiments conducted, [b.] Mean of triplicate repeat.

Table 15: Summary of CV cytotoxicity assays for nitrate ester analogues.

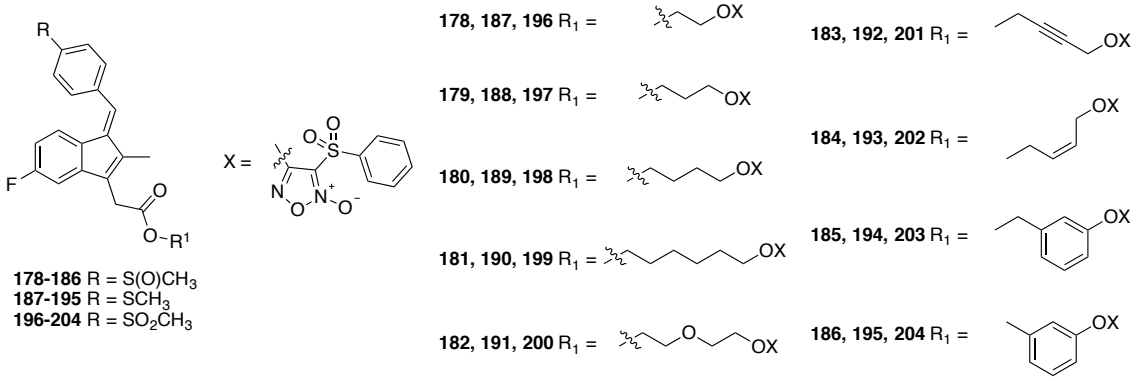
When the range of nitrate esters was expanded to change the length of the linker, and introduce more nitrate ester functionalities, the assays indicated that the majority of the compounds had no significant effect on cell growth. Only two compounds showed a statistically significant reduction in cell growth. These were 2-nitrooxyethyl ester **140**

and dinitrate ester **142**. They had an overall reduction in cell growth to 35.5 % ± 4.2 and 30.2 % ± 4.5, respectively. However, given the overall poor activity of this series, none of the compounds emerged as candidates further testing.

The cytotoxicity of the furoxan analogues **178**-**204** is summarised in Table 16.

In contrast to the nitrate ester analogues, the furoxan analogues were a much more promising series of compounds. At 50 μM, 13 of the 27 compounds tested showed complete cell death after 24 hours. A further 11 compounds displayed a significant reduction in cell growth after 72 hours. Only three compounds had no significant activity.

In the sydnonimine series, of the nine compounds tested, 7 had a significant reduction in cell population after 72 hours. These were amide **210**, the carbamates **227**, **229**, **231** and the ureas **228**, **230**, **232**. In particular, the activity of amide **210**, carbamate **229** and urea **230** was similar to NCX-1102. Amides **221** and **212** showed no statistical reduction in cell growth.

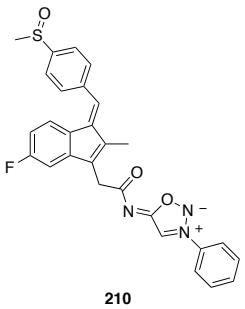


178-186 R = $S(O)CH_3$
187-195 R = SCH_3
196-204 R = SO_2CH_3

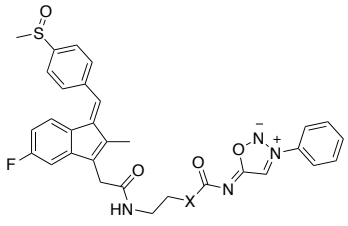
	% Cells alive at time point after treatment at 50 μM[b]		
Entry	24 h	48 h	72 h
DMSO 0.05%[a]	99.1 ± 9.9	100.9 ± 4.9	95.7 ± 7.7
NCX-1102[a]	58.8 ± 8.2	35.5 ± 5.3	24.5 ± 3.9
178	**0.0 ± 0.0**	**0.0 ± 0.0**	**0.0 ± 0.0**
187	**0.0 ± 0.0**	**0.0 ± 0.0**	**0.0 ± 0.0**
196	**0.0 ± 0.0**	**0.0 ± 0.0**	**0.0 ± 0.0**
179	**0.0 ± 0.0**	**0.0 ± 0.0**	**0.0 ± 0.0**
188	**34.9 ± 4.7**	**21.2 ± 4.0**	**15.7 ± 2.5**
197	**0.0 ± 0.0**	**0.0 ± 0.0**	**0.0 ± 0.0**
180	**32.3 ± 4.9**	**21.8 ± 1.8**	**16.5 ± 3.1**
189	**0.0 ± 0.0**	**0.0 ± 0.0**	**0.0 ± 0.0**
198	**67.9 15.4**	**49.9 3.7**	**38.8 ± 3.3**
181	**0.0 ± 0.0**	**0.0 ± 0.0**	**0.0 ± 0.0**
190	**57.2 ± 5.2**	**41.7 ± 8.4**	**29.2 ± 5.3**
199	**42.4 ± 2.4**	**24.2 ± 4.8**	**15.4 ± 1.3**
182	**0.0 ± 0.0**	**0.0 ± 0.0**	**0.0 ± 0.0**
191	**55.2 ± 6.0**	**71.0 ± 5.3**	**50.8 ± 5.7**
200	**0.0 ± 0.0**	**0.0 ± 0.0**	**0.0 ± 0.0**
183	**0.0 ± 0.0**	**0.0 ± 0.0**	**0.0 ± 0.0**
192	**25.4 ± 5.2**	**20.8 ± 2.0**	**15.0 ± 2.9**
201	**36.5 ± 5.1**	**38.0 ± 5.6**	**27.3 ± 6.9**
184	**0.0 ± 0.0**	**0.0 ± 0.0**	**0.0 ± 0.0**
193	**30.6 ± 2.7**	**31.4 ± 6.1**	**20.6 ± 3.3**
202	**0.0 ± 0.0**	**0.0 ± 0.0**	**0.0 ± 0.0**
185	**71.8 ± 4.1**	**56.2 ± 2.3**	**51.9 ± 1.0**
194	101.9 ± 4.0	79.7 ± 4.6	82.7 ± 3.8
203	**88.3 ± 3.2**	**67.7 ± 9.0**	**63.6 ± 0.7**
186	73.9 ± 3.57	67.7 ± 11.1	66.3 ± 10.4
195	81.1 ± 3.1	88.3 ± 5.3	80.6 ± 3.0
204	**0.0 ± 0.0**	**0.0 ± 0.0**	**0.0 ± 0.0**

[a.] Mean of all CV experiments conducted, [b.] Mean of triplicate repeat.

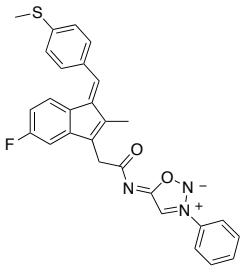
Table 16: Summary of CV data for furoxan analogues.



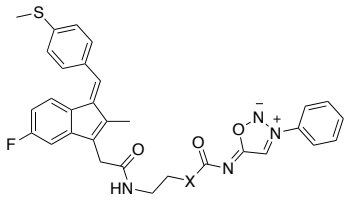
210



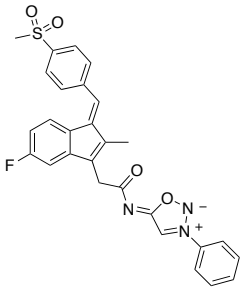
227, X = O
228, X = NH



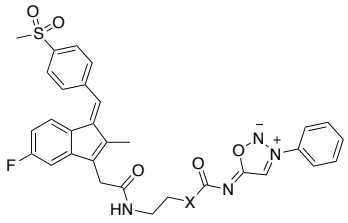
211



229, X = O
230, X = NH



212



231, X = O
232, X = NH

	% Cells alive at time point after treatment at 50 μM[b]		
Entry	24 h	48 h	72 h
DMSO 0.05%[a]	99.1 ± 9.9	100.9 ± 4.9	95.7 ± 7.7
NCX-1102[a]	58.8 ± 8.2	35.5 ± 5.3	24.5 ± 3.9
210	**64.6 ± 16.7**	**29.8 ± 0.5**	**20.6 ± 1.9**
211	89.0 ± 4.9	90.8 ± 2.7	77.0 ± 2.5
212	107.8 ± 21.0	74.5 ± 6.1	81.1 ± 7.4
227	**76.7 ± 0.8**	**46.4 ± 2.7**	**41.4 ± 3.6**
229	**57.7 ± 5.8**	**27.1 ± 1.3**	**17.7 ± 1.3**
231	**76.5 ± 2.0**	**45.9 ± 2.6**	**37.3 ± 4.4**
228	**74.0 ± 4.5**	**46.7 ± 1.2**	**36.3 ± 2.7**
230	**66.5 ± 6.0**	**34.7 ± 1.6**	**21.3 ± 1.8**
232	**86.1 ± 14.2**	**58.2 ± 5.1**	**55.5 ± 2.5**

[a.] Mean of all CV experiments conducted, [b.] Mean of triplicate repeat.

Table 17: Summary of CV data for sydnonimine analogues.

3.4 SUMMARY

In total, fifty-three analogues of sulindac were prepared using three NO-donor functionalities; nitrate esters, furoxans and sydnonimines. Thirty six of these showed a statistical increase in cytotoxic effect relative to control compounds against PC3 cells at a concentration of 50 μM.

After further *in vitro* study (James Black, University of Edinburgh) furoxans **181** and **183** were identified as lead compounds for further study. To this effect, a scale-up for the synthesis of these two compounds will be undertaken to prepare sufficient material for the use in mouse model studies.

CHAPTER FOUR: NITRIC OXIDE-DONATING ANALOGUES OF ABIRATERONE

This chapter describes the development of NO drug conjugates of the drug abiraterone **106**.

It has been known since the work of Charles Huggins in 1941 that prostate cancer growth is driven by androgens.[198] As a result of this, the mainstay treatment of advanced metastatic disease is androgen-ablation therapy as described in Chapter Two.[330] However, within 18-24 months, CRPCa develops driven by adaptive mechanisms distinct from androgen receptor signaling.[331] Upon development of CRPCa the main treatment options of chemotherapy are mainly palliative and the prognosis is terminal. In light of this, new mechanisms for the treatment of CRPCa were sought in the early 1990s to account for adaptive mechanisms of the androgen receptor, in particular, mechanisms indicating hypersensitivity to castration levels of testosterone and those implicating endogenous production of testosterone by the tumour. It was recognised that the ideal therapeutic would give global androgen blockade in any cell capable of biosynthesizing testosterone, and CYP17 was identified as a potential clinical target. CYP17 is an enzyme involved in testosterone biosynthesis from cholesterol **233** (Figure 14).[332] It is a c-P450 responsible for hydroxylation at the 17-position of pregnenolone **234** and progesterone **235** in both the testes and adrenal glands. Following hydroxylation to the steroids **236** and **237**, CYP17 cleaves the acetyl side chain at C-17, generating the 17-keto functionality found in dehydroepiandrosterone **238** and androstenedione **239**.

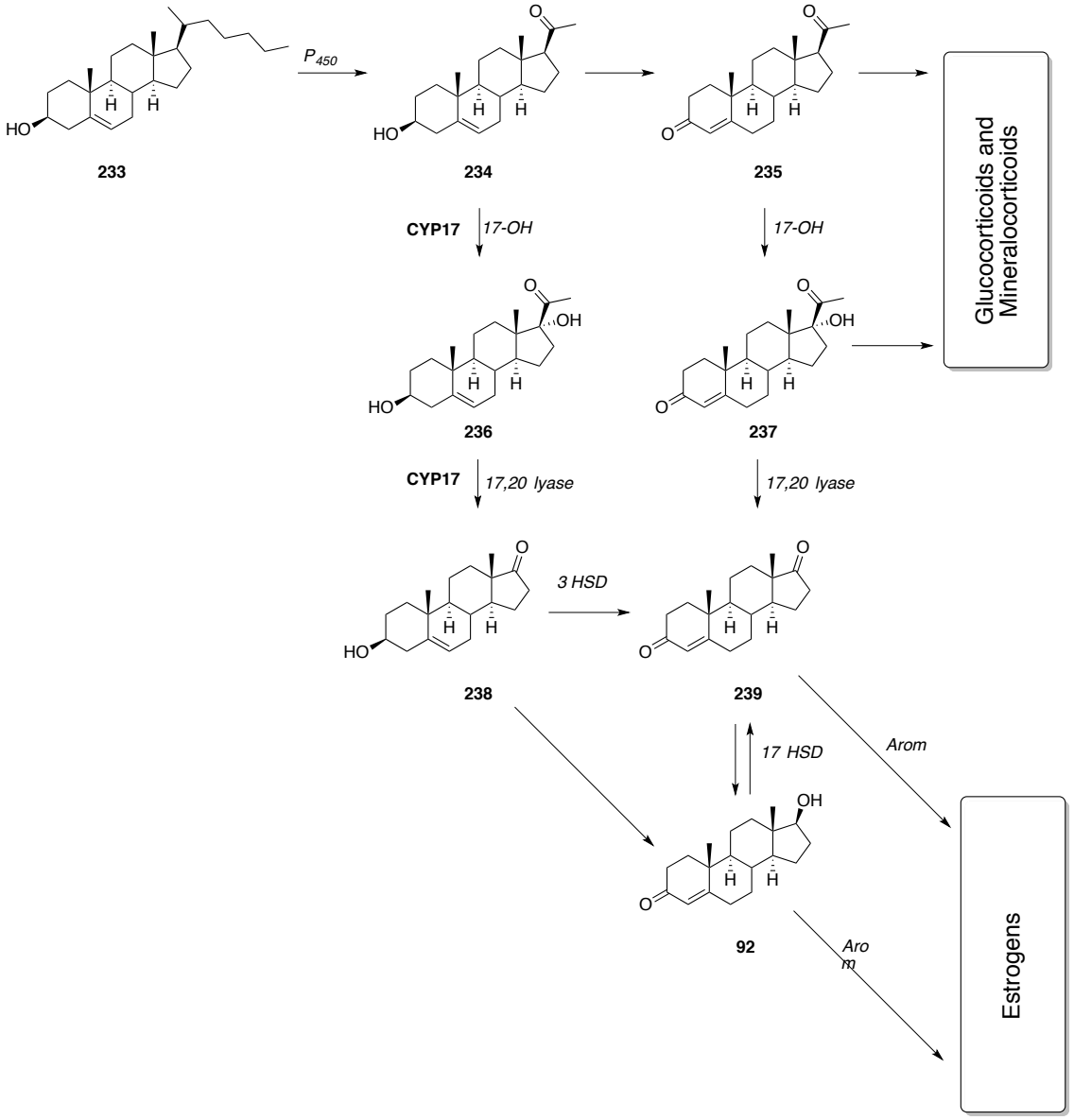
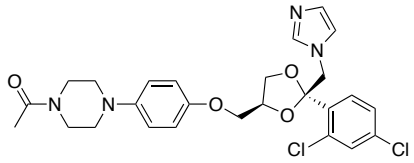


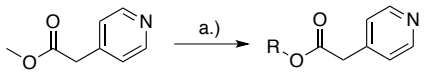
Figure 14: Biosynthetic pathway of cholesterol **233** to testosterone **92**.

These metabolites are intermediates on the pathway to testosterone **92**. Inhibition of this enzyme would clearly prevent the biosynthesis of testosterone in all cells producing it. The antifungal agent ketoconazole **240** has also been shown to inhibit CYP17 when administered in high doses. This compound has been trialled for the treatment of prostate cancer.[333] However, its short half-life *in vivo*, undesirable side-effects and intensive treatment regime ruled ketoconazole out as an effective treatment for CRPCa.



240

Early results from McCague *et al*. identified that 4-pyridylacetic acid esters **241a-e** were effective inhibitors of the hydroxylase-lyase enzyme isolated from rat testes; however, they were unselective, showing potency against the aromatase enzyme also. The esters **241** were prepared by *trans*-esterification of methyl 4-pyridyl acetate **242** with the lithium alkoxide of a range of alcohols (Scheme 59).[334]



242 **241a-e**

Scheme 59: *Reagents and conditions*: a.) ROH, THF, *n*-butyllithium, -78 °C- r.t., 4-16 h, 11-96%. R = see Table 18.

Compounds with 4-substituted cyclohexyl esters, or bulkier lipophilic esters *e.g*. borneyl, isopinocampheyl and adamantyl demonstrated an increased activity for the inhibition of hydroxylase-lyase over the aromatase (Table 18). This selectivity was rationalised as a difference in the hydrophobic pockets available in each active site.[335]

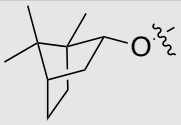
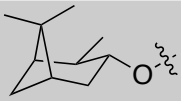
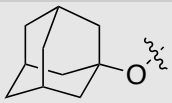
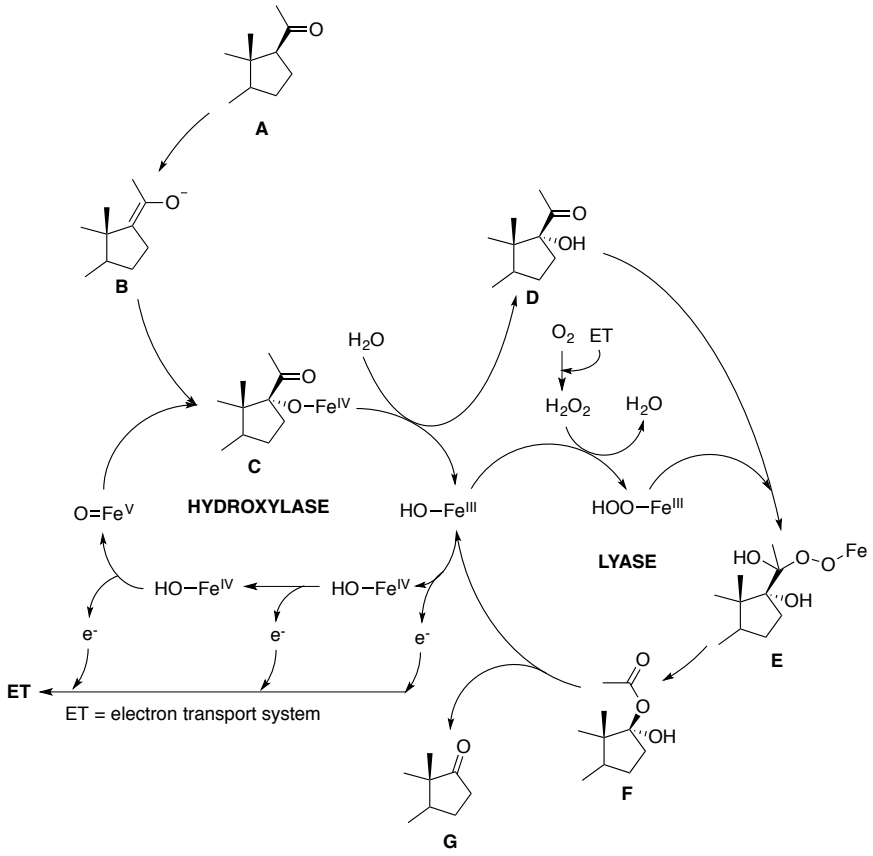
		IC_{50}, μM			
Entry	**R**	**human placental aromatase**	**C_{17-20} lyase**	**17α-hydroxylase**	**ratio of lyase/aromatase potency**
241a		0.30	20	15	67
241b		0.15	4.4	4.2	29
241c		0.097	2.2	1.5	23
241d		0.096	1.8	1.7	19
241e		0.089	0.61	0.56	6.9

Table 18: Activities of 4-pyridyl acetic acid esters **241a-e** against rat C17-20 lyase and 17α-hydroxylase.[334]

Molecular modelling has indicated that bulky esters which display increased lyase and hydroxylase inhibition are acting as bioisosteres of a steroid motif and could enter a larger hydrophobic pocket than is present in the aromatase.[335]

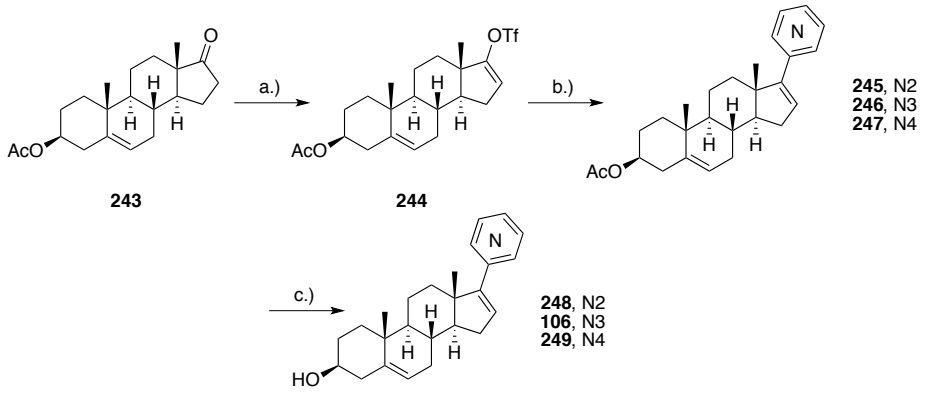
This led to the preparation of steroids substituted at the 17-position with pyridyl groups. It was postulated from molecular modelling[335] that the pyridyl nitrogen coordinates the iron in the haem cofactor and that a nitrogen in the 2- or 3-position would give optimum coordination geometry. The haem is critical for both the activity of the hydroxylase and lysase. The proposed mechanism is shown in Scheme 60.



Scheme 60: Mechanism of CYP17 oxidation. Adapted from Potter *et al.*[291]

A key aspect of the process is a Baeyer-Villeger rearrangement of peroxyhemiacetal **D** to liberate the haem species and generate a 17-*O*-acetyloxy steroid **F**.[336] This decomposition occurs with the loss of acetic acid to generate the 17-keto functionality **G**.[336] Coordination of the pyridyl nitrogen as a sixth ligand to the haem iron prevents the access of molecular oxygen and this inhibits both steps of the enzyme, arresting androgen biosynthesis.

A series of 17-pyridyl steroids was designed and prepared by Potter *et a*l. based on the pregnenolone skeleton, the preferred substrate for the human enzyme.[337] The pyridyl nitrogen was placed sequentially at the 2-, 3- and 4-positions. The three target steroids were prepared from dehydroepiandrosterone 3-acetate (Scheme 61). Preparation of the relevant enol triflate and subsequent cross-coupling with the desired pyridyl unit furnished the steroid acetates. These were then deprotected under basic conditions.[291]



Scheme 61: *Reagents and conditions*: 2,6-di-*tert*-butyl-4-methylpyridine, Tf_2O, CH_2Cl_2, 58%; b.) 2-position: 2-pyridylzinc chloride, bromo(isoprenyl)-*bis*(triphenylphosphine)palladium (II), THF, **245** = 74%; 3-position: diethyl(3-pyridyl)borane, $Pd(PPh_3)_2Cl_2$, THF, 2 M Na_2CO_3, **246** = 84%; 4-position: lithium trimethoxy(4-pyridyl)boronate, $Pd(PPh_3)_2Cl_2$, THF, 2 M Na_2CO_3; c.) NaOH, H_2O, MeOH, **248** = 78%, **106** = 79%, **249** = 53 % (over two steps).

The ability of **106**, **248**, **249** to inhibit CYP17 lyase and hydroxylase activities was assayed using ketoconazole as a positive control. Steroid **106** emerged the most potent activity *in vitro* showing an IC_{50} of 2.9 nM and 4 nM against the lyase and hydroxylase respectively, a greater than 10-fold increase in activity compared to ketoconazole. Compound **246**, the acetate protected steroid **106**, was tested *in vivo* in mice; and it displayed an excellent ability to reduce testosterone to an undetectable level, as well as reducing the mass of androgen dependent organs.[291]

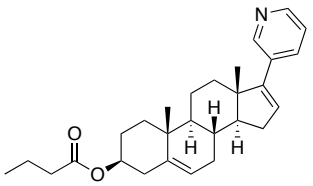
Compound **106**, now referred to as abiraterone **106** progressed into human clinical trials and was recently approved for use by the NHS for the treatment of advanced CRPa. Abiraterone **106** is the first in a new class of drugs to be used for the treatment of CRPa. While the clinical trial data demonstrated that abiraterone **106** is effective for the treatment of CRPa, the median radiographic progression-free time was found to be 5.6 months, with a median overall survival of 3.9 months (compared to placebo).[338] In

addition, 3-50% of patients exhibited a primary resistance to abiraterone **106** after beginning treatment and all patients ultimately exhibited resistance.[339]

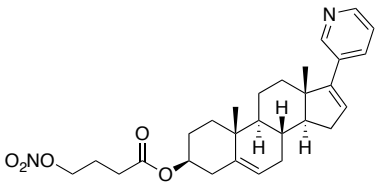
As such, there is potential to improve the activity of the drug and this is the focus of this aspect of the thesis. Following on from the reported bioactivity of nitric oxide in the sulindac **112** compounds, a series of NO-donating analogues of abiraterone **106** was proposed. Abiraterone **106** is delivered clinically as the acetate prodrug **246**, requiring an *in vivo* hydrolysis to release the drug. As such, by changing the acetate ester to an ester linked to an NO donating unit, abiraterone **106** and the NO functionality should reasonably be targeted to the site of hydrolysis.

4.1 AIMS

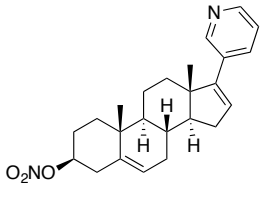
The aim of this aspect of the research was to prepare a series of NO-donating analogues of abiraterone **106**. The new constructs will be assayed along with an appropriate control. An immediate objective was prepare a series of NO-donating carboxylic acids and the appropriate abiraterone esters. Thus the structures **250**-**254** emerged as synthesis targets.



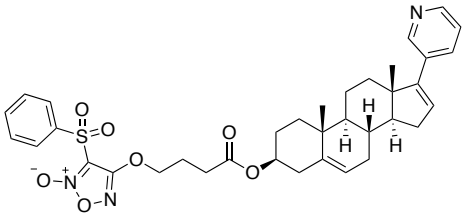
250



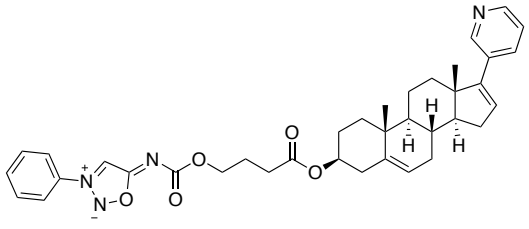
251



252



253

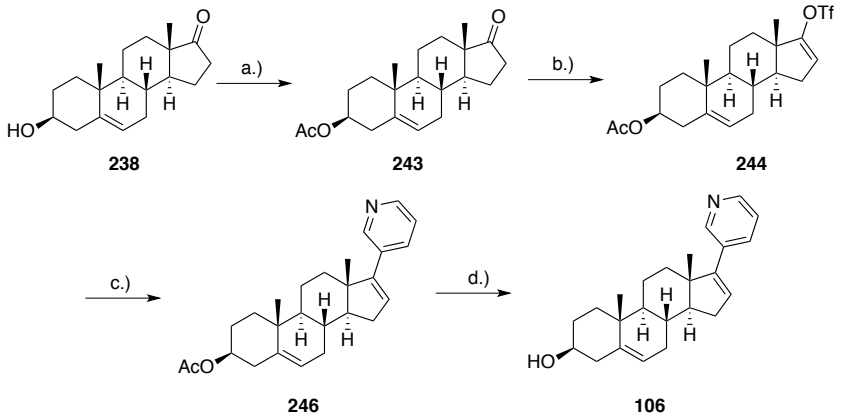


254

.

4.2 RESULTS AND DISCUSSION

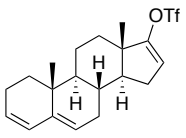
The synthesis of abiraterone **106** reported by Potter *et al*. starts from dehydroepiandrosterone 3-acetate **243**.[291] As this material was unavailable, commercially available dehydroepiandrosterone **238** was chosen as the starting material. This was converted to abiraterone in four-steps based on known literature procedures.[291] This synthesis was accomplished in a 61% overall yield (Scheme 62).



238 **243** **244** **246** **106**

Scheme 62: *Reagents and conditions*: a.) Ac_2O, $BF_3.(OEt)_2$, CH_2Cl_2, r.t., 4 h, quant.; b.) 2,6-di-*tert*-butyl-4-methylpyridine, Tf_2O, CH_2Cl_2, r.t., 18 h, 58%; b.) diethyl(3-pyridyl)borane, $Pd(PPh_3)_2Cl_2$, THF, 2 M Na_2CO_3, reflux, 1.5 h, 87%; c.) 10% KOH in MeOH, MeOH, 0 °C, 1 h, quant.

Acetylation at the 3-position with acetic anhydride and catalytic $BF_3.(OEt)_2$ furnished the protected acetate **243** in quantitative yield (Scheme 62). Treatment with trifluoromethanesulfonic anhydride using 2,6-di-*tert*-butyl-4-methyl pyridine as a base furnished the desired enol triflate **244** (58%) (Scheme 62). A quantity of the undesired triene **255** was also isolated along with another minor impurity. This accounted for approximately 10% of the mass balance isolated.



255

Palladium catalysed Suzuki cross-coupling using $Pd(PPh_3)_2Cl_2$, diethyl(3-pyridyl)borane and aqueous Na_2CO_3 in THF provided the desired abiraterone acetate **246** (Scheme 62). This was deprotected based on an alternative procedure used to prepare a benzimidazole analogue of abiraterone.[340] Treatment with methanolic KOH in methanol furnished abiraterone **106** in quantitative yield, without the need for chromatography (Scheme 62).

The ^{13}C NMR spectrum of enol triflate **244**, abiraterone acetate **246** and abiraterone **106** are reported for the first time due to incomplete data in the literature. In addition, the use of HSQC correlation spectroscopy allowed for the steroid backbone protons to be unambiguously assigned in the ^{1}H and ^{13}C NMR spectrum; for example Figure 15.

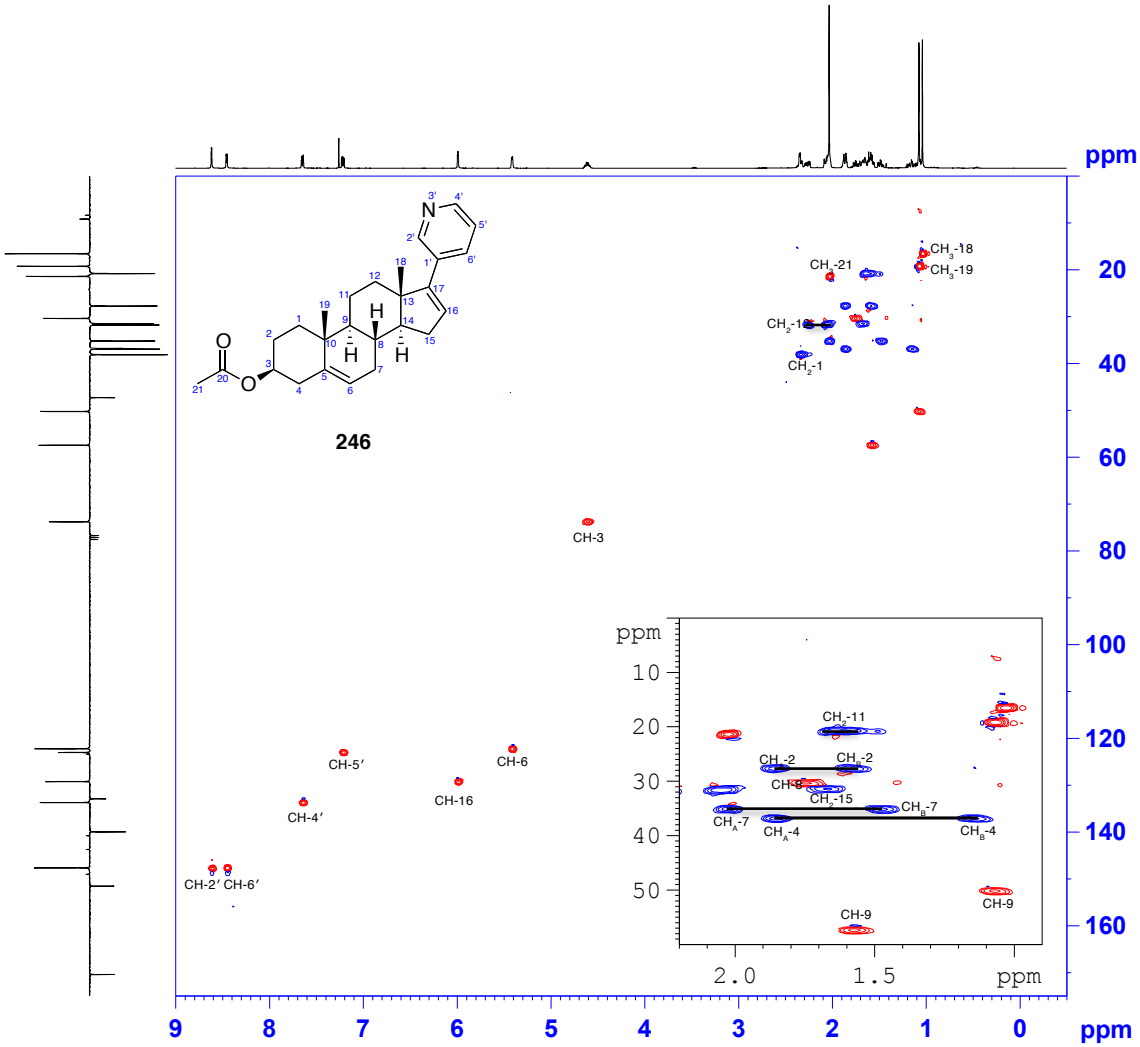
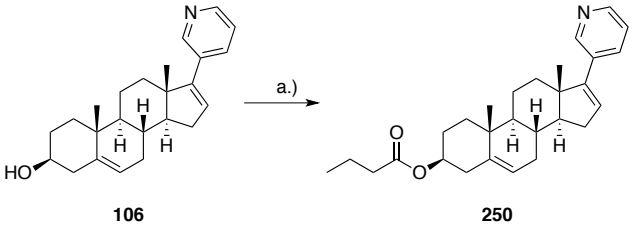


Figure 15: HSQC spectrum of abiraterone acetate **246** (^{1}H 500 MHz, ^{13}C 75 MHz, $CDCl_3$)

Esterification of abiraterone **106** with butyric acid furnished the butyrate ester in 79% yield as a white solid (Scheme 63). This material was prepared as a control compound as a non-NO donating ester.

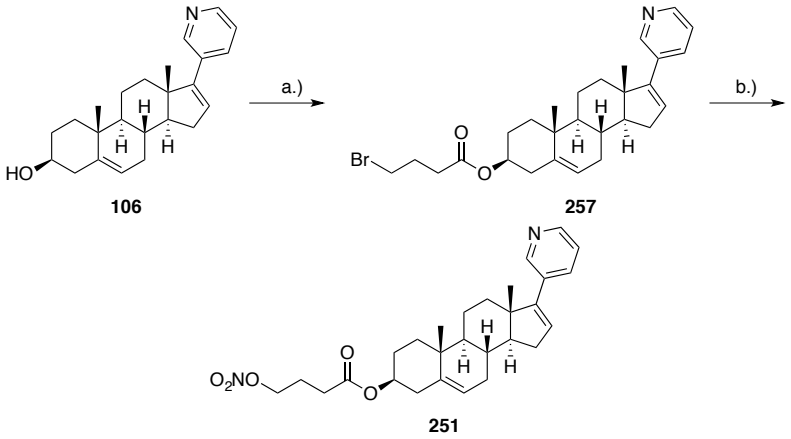


106 **250**

Scheme 63: *Reagents and conditions*: a.) Butyric acid, EDCI.HCl, DMAP, CH_2Cl_2, r.t., 18 h, 79%.

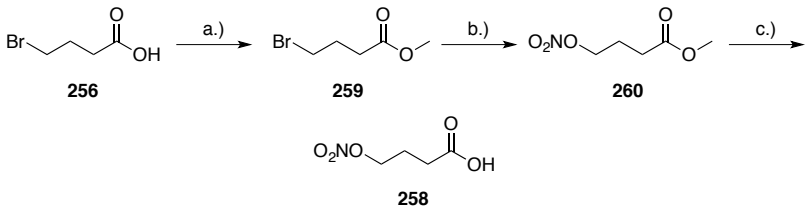
This ester **250** has an alkyl chain, which does not link to an NO-donating functionality. On the other hand the three proposed targets **251**, **253** and **254** have an alkyl linker which terminates with an NO-donating functionality. Ester **250** will serve as a control with a longer alkyl chain to that of acetate **246**.

Target **251** combined the nitrate ester with abiraterone **106**. The initial synthesis of **251** involved a two-step process. Initially abiraterone **106** was esterified using 4-bromobutyric acid **256** to furnish the bromobutyrate **257**. This was followed by bromide substitution with nitrate (Scheme 64).



Scheme 64: *Reagents and conditions*: a.) 4-Bromobutyric acid, EDCI.HCl, DMAP, CH_2Cl_2, r.t., 18 h, 79%; b.) $AgNO_3$, CH_3CN, 90 °C, 18 h, 18%.

Treatment of **257** with silver nitrate resulted in a low conversion to the nitrate ester by ^{1}H NMR. Heating the reaction to 90 °C resulted in the complete consumption of starting however, the isolated yield was only 18%. Disappointingly, elemental analysis showed that the material was impure. This is likely to be due to residual silver impurities in the product. Given the low yield of nitrate **251,** an alternative route was devised. This involved coupling of 4-(nitrooxy)butyric acid **258** to abiraterone **106**. 4-(Nitrooxy)butyric acid **258** was prepared from 4-bromobutyric acid (Scheme 65).

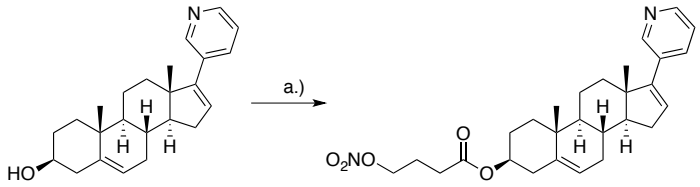


256 **259** **260** **258**

Scheme 65: *Reagents and conditions*: a.) AcCl, MeOH, 0 °C to r.t., 18 h, quant.; b.) $AgNO_3$, CH_3CN, 90 °C, 18 h, 97%; c.) LiOH, MeOH, 5 °C, 18 h, 77%.

4-Bromobutyric acid **256** was converted to the corresponding methyl ester **259**. Treatment with silver nitrate furnished methyl 4-(nitrooxy)butyrate **260** in good yield. While this material was of relatively high purity, purification by chromatography was carried out to remove any residual silver salts. Finally, saponification of the methyl ester **260** with aqueous lithium hydroxide at 5 °C provided the required 4-(nitrooxy)butyric acid **258** in 77% yield.

Finally, coupling of abiraterone **106** with 4-(nitrooxy)butyric acid **258** using EDCI and stoichiometric DMAP gave the desired 4-(nitrooxy)butyrate ester **251** in 95% yield (Scheme 66).

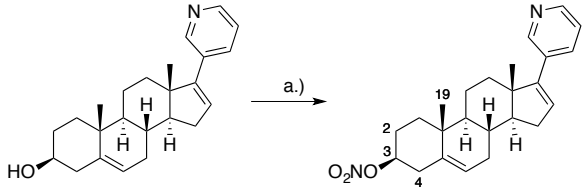


106 **251**

Scheme 66: *Reagents and conditions*: a.) **258**, EDCI.HCl, DMAP, CH_2Cl_2, r.t., 4 h, 95%.

A second nitrate ester **252** was prepared. This was the direct *O*-nitrate ester at the 3-position of abiraterone **106**. This was accomplished using zinc nitrate and EDCI.HCl, based on conditions reported by Sarma[341] for the formation of the 3-*O*-nitrate of

cholesterol **233**. The reaction occurs with retention of configuration at C3. The reaction proved straight forward with abiraterone **106**, and gave the desired nitrate ester **252**, which was isolated in 35% yield (88% b.r.s.m).

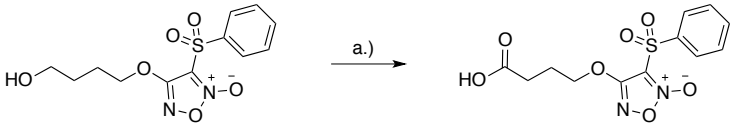


106 **252**

Scheme 67: *Reagents and conditions*: a.) $Zn(NO_3)_2.(H_2O)_6$, EDCI.HCl, DMAP, CH_3CN:CH_2Cl_2 (1:1), r.t., 18 h, 35%.

Steroid **252** shows a ^{1}H NMR NOE between CH-3 and the protons in the CH_2-2 and CH_2-4 positions: There is no NOE correlation to CH_3-19, which would be expected if there had been an inversion of the configuration upon nitrate ester formation the stereochemistry of this position had been inverted.

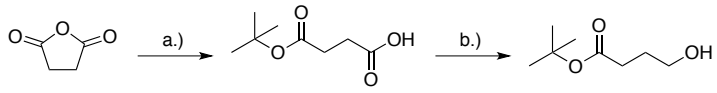
In order to prepare furoxan target **253**, carboxylic acid **261** was prepared from alcohol **167** following a literature procedure.[342] The preparation of **167** is reported *vide supra* in Chapter Three.



167 **261**

Scheme 68: *Reagents and conditions*: a.) 2.5 M Jones reagent, acetone, 0 °C to r.t., 0.5 h, 23%.

Oxidation of **167** was accomplished with Jones reagent, however on work up the desired acid was isolated in a low, 23% yield. As a result, an improved method was sought. To this end, *tert*-butyl 4-hydroxybutanoate **262** was prepared in two steps from succinic anhydride **263** (Scheme 69).

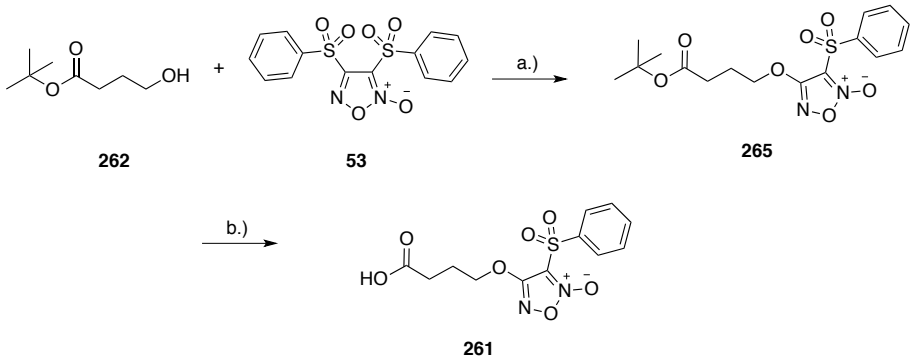


263 **264** **262**

Scheme 69: *Reagents and conditions*: a.) *tert*-BuOH, Et_3N, HOSu, DMAP, toluene, reflux, 18 h, 77%; b.) $BH_3.Me_2S$, r.t., 18 h, quant.

Ring opening of succinic anhydride **263** with *tert*-butanol furnished the *tert*-butyl hemi succinate **264** in 77% yield after recrystallization from petroleum ether (Scheme 69). Selective reduction of the carboxylic acid was achieved using $BH_3.Me_2S$ to provide the desired alcohol **262** in quantitative yield (Scheme 69).

Sulfonyl substitution with alcohol **262** onto *bis*(phenylsulfonyl)furoxan **53** using DBU, furnished the desired ether **265** in 95% yield (Scheme 70). Deprotection with trifluoroacetic acid gave carboxylic acid **261** in quantitative yield. (Scheme 70). Although this protocol involved an extra two steps, the route avoids the use of toxic chromium, and provided **261** in a 73% overall yield. This can be compared very favourably with 11.5% over two steps using the Jones oxidation.

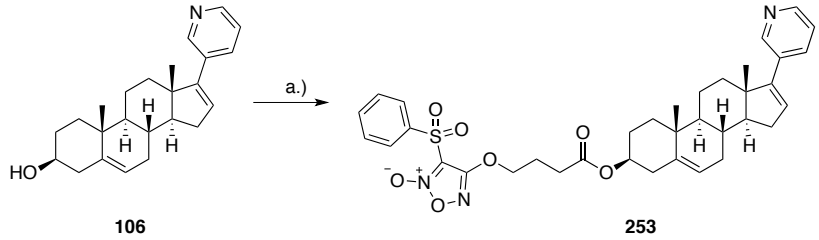


262 **53** **265**

261

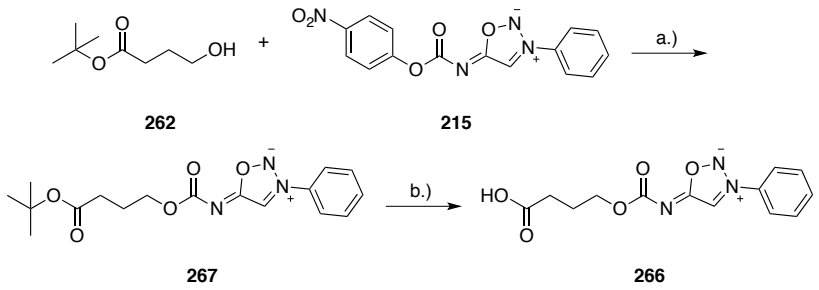
Scheme 70: *Reagents and conditions:* a.) DBU, CH_2Cl_2, r.t., 2 h, 95%**;** b.) TFA, CH_2Cl_2, r.t., 16 h, quant.

With carboxylic acid **261** in hand, attention was drawn to preparing target **253**. Esterification using previously established conditions, furnished the desired abiraterone ester **253** in 92% yield (Scheme 71).



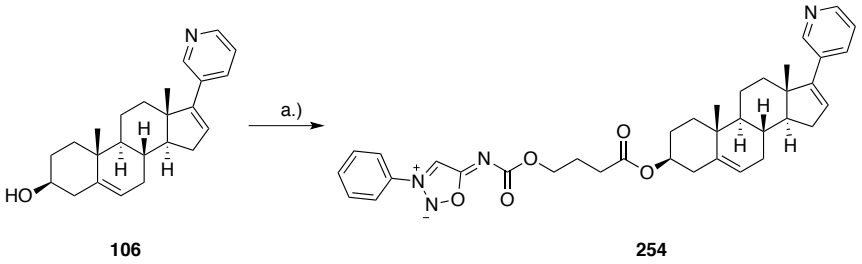
Scheme 71: *Reagents and conditions*: a.) **261**, EDCI.HCl, DMAP, CH_2Cl_2, r.t., 18 h, 92%.

A sydnonimine carboxylic acid **266** was also prepared using the methodology developed in Chapter Three.



Scheme 72: *Reagents and conditions*: a.) CH_3CN, reflux, 18 h, 70%; b.) TFA, CH_2Cl_2 r.t., 18 h, quant.

Acylation of alcohol **262** with *p*-nitrophenolate **215** resulted in the *tert*-butyl protected sydnonimine carbamate **267** in 70% yield. Deprotection of the *tert*-butyl ester with trifluoroacetic acid furnished the sydnonimine acid **266** in quantitative yield. Coupling of this acid to abiraterone **106** proved to be sluggish, requiring 48 hours to proceed to completion, however the desired ester **254** was isolated in an 88% yield.



Scheme 73: *Reagents and conditions*: a.) **266**, EDCI.HCl, DMAP, CH_2Cl_2, r.t., 48 h, 88%.

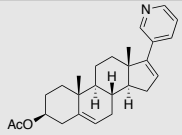
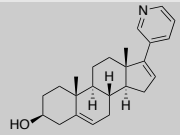
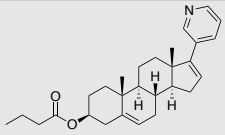
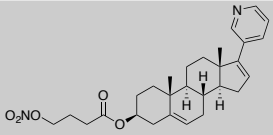
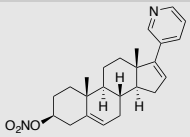
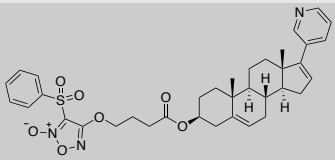
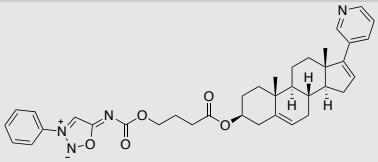
4.3 CYTOTOXICITY RESULTS

In total, seven abiraterone based compounds were evaluated for cytotoxicity against PC3 cells at the University of Edinburgh, using the same assay described in Chapter Three. Abiraterone **106** and abiraterone acetate **246** were used as positive controls The esters **251**-**254** were all designed as NO-donating compounds. Butyrate ester **250** was tested as a further control as a longer chain, non-NO-donating analogue.

The crystal violet assay was carried out by Mr James Black of the University of Edinburgh. The compounds were screened against PC3 cells under normoxic conditions at a concentration of 50 μM. The results are summarised in Table 19.

Of the seven compounds screened, five showed a statistically significant reduction in cell growth compared to the DMSO control. Of these, two were abiraterone acetate **246** and abiraterone **106**. A significant level of activity was expected for these two molecules based on their already established clinical use. Of the NO-donating series, nitrate ester **251** and furoxan **253** showed significant activity. Furoxan **253** showed a higher activity than NCX-1102 **111**. Interestingly butyrate ester **250** was cytotoxic indicating that longer esters (than acetate **246**) also increase activity.

These initial results suggest that NO-donating analogues of abiraterone are active against PC3 cells with furoxan **253** emerging as a lead compound for further investigation.

	% Cells alive at time point[b]		
Entry	24 h	48 h	72 h
DMSO 0.05%[a]	99.1 ± 9.9	100.9 ± 4.9	95.7 ± 7.7
NCX-1102 111[a]	58.8 ± 8.2	35.5 ± 5.3	25.5 ± 3.9
 **246**	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
 **106**	54.6 ± 8.5	57.7 ± 14.1	34.7 ± 4.0
 **250**	68.1 ± 13.9	59.0 ± 14.0	32.8 ± 4.0
 **251**	76.6 ± 8.5	56.6 ± 13.1	35.6 ± 3.34
 **252**	86.3 ± 7.4	92.2 ± 15.0	70.6 ± 2.8
 **253**	48.0 ± 12.6	27.5 ± 10.9	12.8 ± 3.4
 **254**	89.8 ± 12.5	99.1 ± 16.3	83.8 ± 12.4

[a.] Mean of all CV experiments conducted, [b.] Mean of triplicate repeat.

Table 19: Summary of CV results for abiraterone series.

4.4 SUMMARY

Abiraterone **106** was prepared in four steps from the commercially available steroid **238**. A series of NO-donating carboxylic acids were prepared and abiraterone analogues prepared using an EDCI.HCl coupling to furnish the esters. In total, four analogues of abiraterone were prepared using three NO-donor functionalities; nitrate esters, furoxans and sydnonimines. Two of these showed a statistical increase in cytotoxic effect relative to control compounds against PC3 cells at a concentration of 50 μM.

Further *in vitro* study (James Black, University of Edinburgh) is underway to investigate the activity of the compounds in the LnCAP cell line, a hormone-sensitive prostate cancer cell line.

CHAPTER FIVE: TARGETING NITRIC OXIDE RELEASE WITH AMINO ACIDS AND PEPTIDES

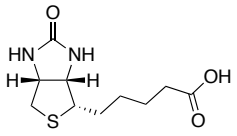
This Chapter details the development of NO-releasing amino acids, and their applications in bioactive peptide assembly.

The development of new drugs by medicinal chemists hinges on their ability to recognise and capitalise on the molecular recognition of a small molecule with the desired target. By identifying key molecular interactions between biological substrates and designed molecules, the potency and specificity of new molecular entities is enhanced.

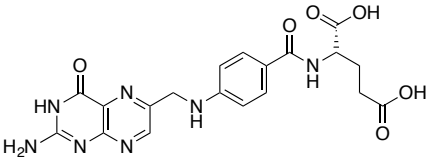
This design process is extensive, involving organic synthesis, *in silico* modeling,[343] and more recently, library and fragment-based screening.[344]

In contrast, molecular recognition in biology has evolved a series of highly conserved motifs which have begun to be exploited by medicinal chemists as a way of targeting drug delivery, often in oncology therapies. Many drugs are effective, however their related toxicity to healthy tissues reduces their clinical effectiveness due to adverse side effects.[345] By developing a conjugate between a molecular recognition motif and the drug, the altered vector can help to circumvent the associated side effects.

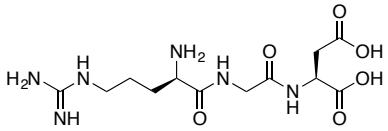
Many of these motifs are highly specific for a distinct cellular target, such as a receptor or protein surface, and they display a high affinity and specificity *in vivo*. Examples of these include biotin **267**,[346] folate **268**,[347] octreotide (OctR) **269**[348] and Arg-Gly-Asp (RGD) **270**,[349] as well as larger units such as antibodies, oligonucleotide sequences[350] and antibody-fusion proteins.[351]



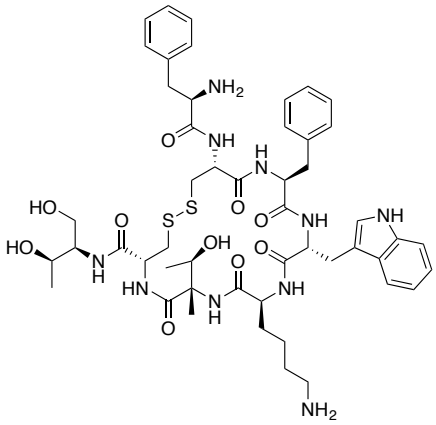
267



268

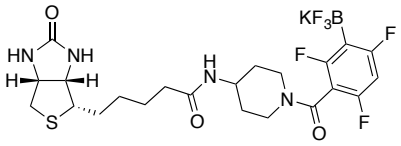


270



269

These recognition motifs have been exploited for a large variety of uses in chemical biology. For example, Ting *et al*. demonstrated that biotinylation of an ^{18}F labelled aryltrifluoroborate ($ArBF_3^-$) ligand **271** was able to localise the distribution of the ligand to bladder, stomach, liver and salivary glands in mice; in contrast to the localization of free fluoride to the bone.[352]



271

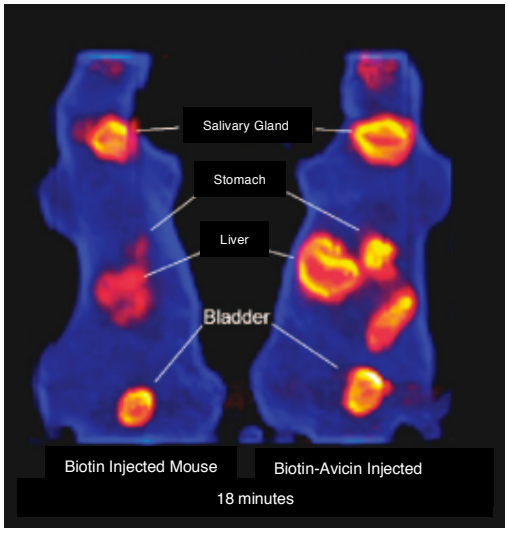
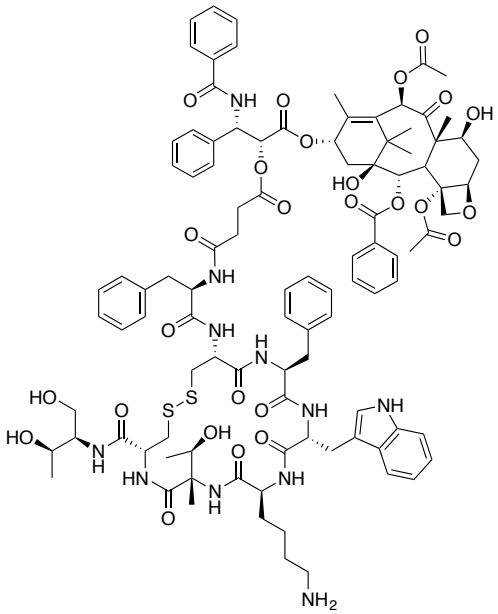


Figure 16: Reprinted (adapted) with permission from Ting *et al. J. Am. Chem. Soc.*, **2008**, *130*, 12045-12055. Copyright 2008 American Chemical Society.

Peptide based recognition units OctR **269** and RGD **270** have been used as targeting anchors for drug conjugation.[348,353] Pacitaxel was conjugated to OctR through a succinate linker to develop an OctR-Taxol conjugate **272**.[348]



272

Conjugate **272** was shown to be exclusively toxic to cells expressing endogenous somatostatin G-protein coupled receptors (SSTRs), with reduced toxicity to low SSTRs expressing cells, demonstrating cell selectivity and targeting of taxol.[348]

The RGD sequence is a cell adhesion sequence discovered in fibronectin by Pierschbacher & Ruoslahti,[354] which binds to receptor proteins called integrins. After much scepticism it was confirmed that this three amino acid sequence plays a central role in adhesion biology, with RGD being the prototypical adhesion signal. As such, it has been widely exploited in medicinal chemistry as a targeting motif. Cell adhesion plays a crucial role in a variety of basic biological processes, such as cell migration, cell anchorage, proliferation, differentiation and apoptosis.[355] In cancer, changes in cell adhesion have roles in metastasis, tumour angiogenesis and in the evasion of apoptosis.[356]

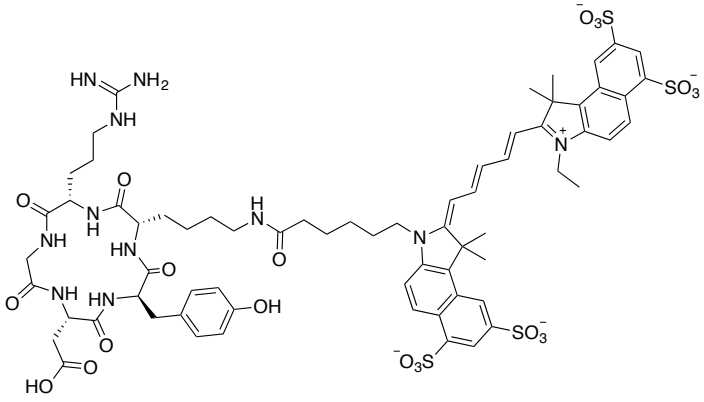
The RGD sequence can easily be introduced into peptides to develop synthetic RGDs, which can act in two different ways:[357] On the surface of cells, the RGD sequence promotes cell adhesion, and in solution, they act as a decoy, preventing adhesion by binding to soluble integrin fragments and disrupting cell signalling..[357] Up to twelve of the twenty known integrins recognise the RGD sequence in their respective ligands, and the RGD sequence has been identified in a large number of adhesion proteins.[349] Tumour cell expression of the integrins $\alpha_v\beta_3$, $\alpha_v\beta_5$, $\alpha_5\beta_1$, $\alpha_6\beta_4$ and $\alpha_v\beta_6$ is correlated with cancer progression and has led to intense study.[358] For example, the $\alpha_v\beta_3$ is widely expressed in the blood vessels of human tumours, as confirmed by biopsy, however, it is not expressed in the vessels of normal tissue. The over expression of $\alpha_v\beta_3$ has been identified to be correlated to a number of disease markers.

In breast cancer and prostate cell carcinoma, $\alpha_v\beta_3$ integrin expression is correlated with bone metastasis due to an association between integrins and the processes of cell migration and adhesion involving osteopontin,[359–361] a bone sialoprotein found in bone tissue. The $\alpha_v\beta_3$ integrin is overexpressed in the invasive margins of glioblastoma in conjunction with increased levels of fibronectin,[361] it is associated with enhanced cell motility and resistance to apoptosis.[361] In pancreatic cancer, $\alpha_v\beta_3$ is associated with lymph node metastasis.[362]

Because of the widespread overexpression of the $\alpha_v\beta_3$ integrin in cancer, the RGD sequence has been exploited as a mechanism to target cancer cells. For example, coinjection of RGD peptides with tumour cells into the blood stream prevents the subsequent growth of tumour nodules in other tissues,[363] effectively acting as an anti-metastatic agent. Spontaneous metastasis of tumours has also been halted using this type of treatment.[364] RGD peptides have been shown to inhibit tumour cell invasion *in vitro*.[365]

Development of synthetic RGD peptides has added a new dimension to this therapy. By adding a cytotoxic or radiolabelled group to a RGD peptide, this warhead can be delivered effectively to antigenic cells.[356]

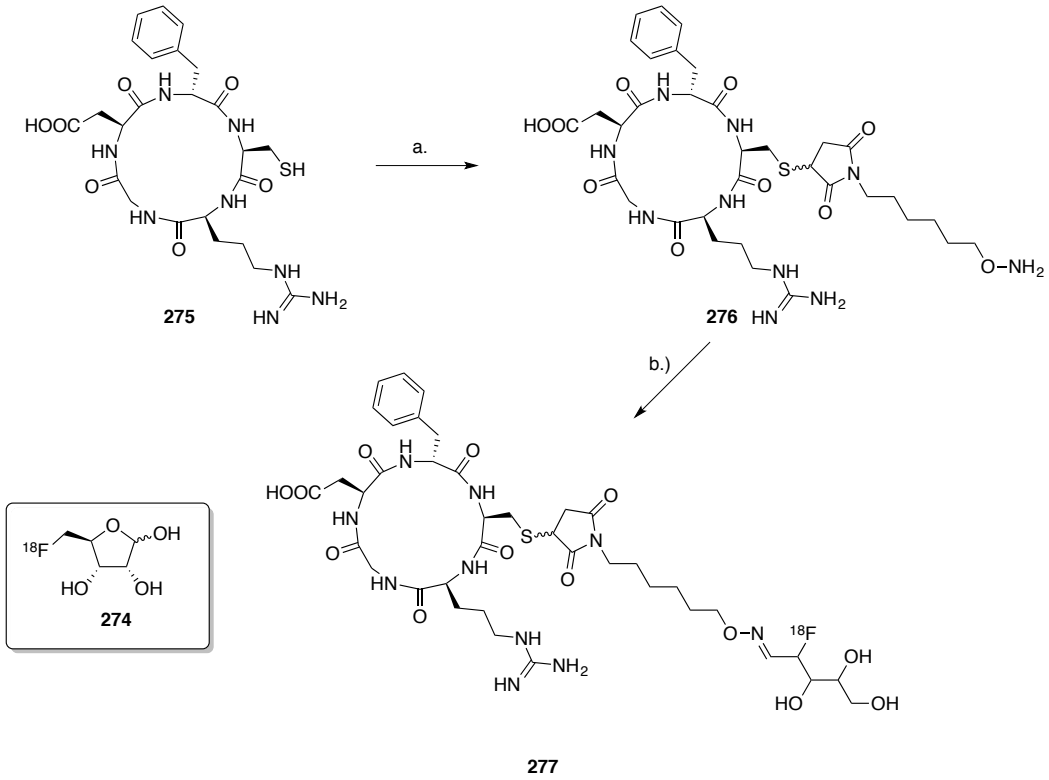
One example of the use of this is in imaging technologies. Cheng *et al.* were able to track $\alpha_v\beta_3$ expression in living xenograft mice (U87MG) using an RGD conjugate **273** with a near-infrared fluorescent (NIRF) dye, Cy5.5.[366]



273

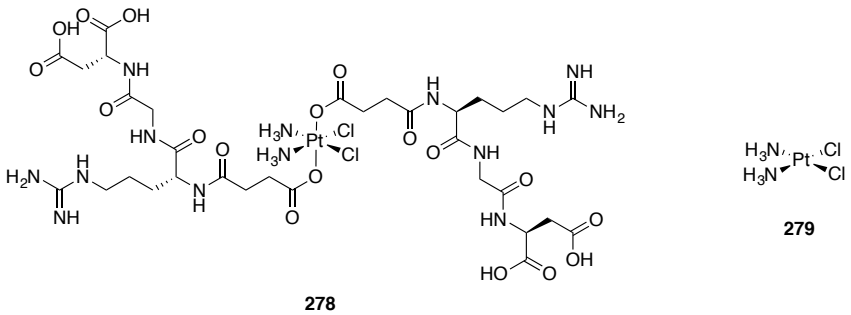
The mice were injected with human glioblastoma cells at 4-6 weeks of age, and subsequently imaged at 14-21 days after implant, when the tumour had reached a diameter of 400-600 mm. Following injection with RGD-NIRF dye **273**, they were able to observe $\alpha_v\beta_3$ distribution using a tungsten-halogen lamp to excite the dye at 610-665 nm, and the dye showed a high selectivity for tumour tissue in living *in vivo* model and later dissection analysis.[366]

Synthetic work in the O'Hagan laboratory has been focussed on the use of RGD peptides as a tool in positron emission tomography using 18-fluorine. The fluorosugar 18-FDR **274** has been successfully bioconjugated to c(RGDfC) **275** using a cysteine maleimide conjugation to give **276** and then oxime trapping of the fluorosugar to give FDR-conjugate **277** (Scheme 74).[367]



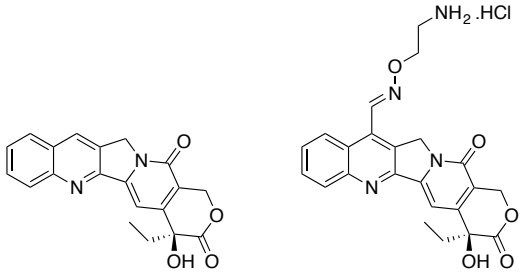
Scheme 74: *Reagents and conditions:* a.) aminooxyhexylmaleimide, H_2O, r.t., 20 min; b.) **274**, NH_4OAc buffer, r.t., 30 min, 86% ("cold reaction").[367]

The exquisite selectivity of the RGD sequence is also evident in the delivery of chemotherapy agents. Mukhopadhyay *et al*. developed a series of platinum (IV)-RGD complexes, *e.g*. **278** which were specifically cytotoxic to cell lines containing the $\alpha_v\beta_3$ and $\alpha_v\beta_5$ integrins, with toxicities approaching that of cisplatin **279**.[368]



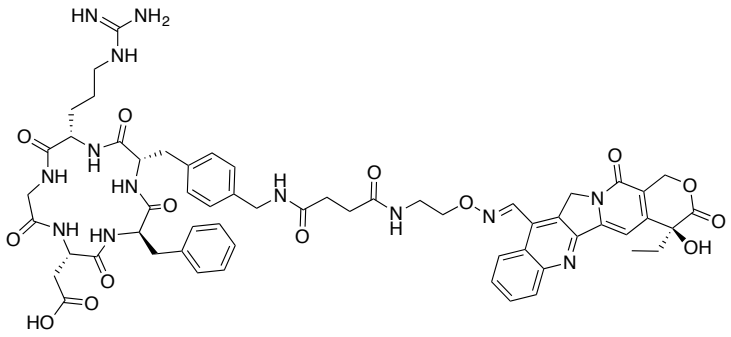
It was shown that the linear RGD containing Pt(IV) complexes *e.g.* **278** were equally effective as the cyclic RGD peptides in cytotoxicity and specificity.[368]

Dal Pozzo *et al*. generated a series of RGD conjugates with the quinolone alkaloid camptothecin **280**, a known inhibitor of the DNA enzyme, topoisomerase I.[369] In preliminary clinical trials, camptothecin displayed good anticancer activity, but had poor solubility and adverse side effects. As such, namitecan **281**, a 7-position modified hydrophilic analogue, was developed.[353]



280 **281**

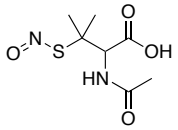
The synthetic RGD-camptothecin analogues, *e.g*. **282** were both cytotoxic and selective to cells expressing $\alpha_v\beta_3$ and $\alpha_v\beta_5$ integrins, identifying this strategy as a possible mechanism to improve the selectivity of camptothecin analogues.[353]



282

In the light of the recent developments with the RGD motif it became an objective to explore the preparation of a series of NO-donating amino acids, which could be incorporated into the appropriate peptide constructs.

One widely used NO-donating amino acid is SNAP **29**.[89] SNAP **29** is an *S*-nitrosothiol and has long had use in biochemistry as a positive control for NO release.



29

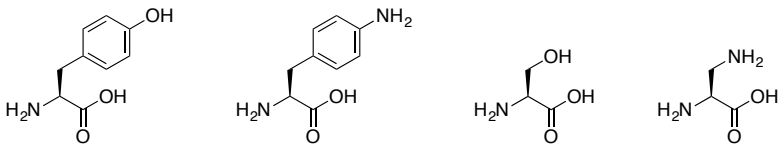
Its stability is solution is dependent of temperature, buffer composition and metal content, and as such, conditions can be tuned to alter its half-life from seconds to hours. Due to this instability SNAP **29** is not used clinically.

Amino acids provide the basic building blocks for many aspects of synthetic chemistry, from natural product total synthesis, to combinatorial synthesis of oligopeptides. By developing a number of amino acids with the potential for orthogonal protecting group manipulation it was envisaged that they could be used in chemical biology as targeted NO-donors, in combination with known recognition sequences.

To develop these amino acids, it was chosen to chemically modify naturally occurring amino acids, in this way the absolute configuration would be immediately introduced without the need for an asymmetric synthesis.

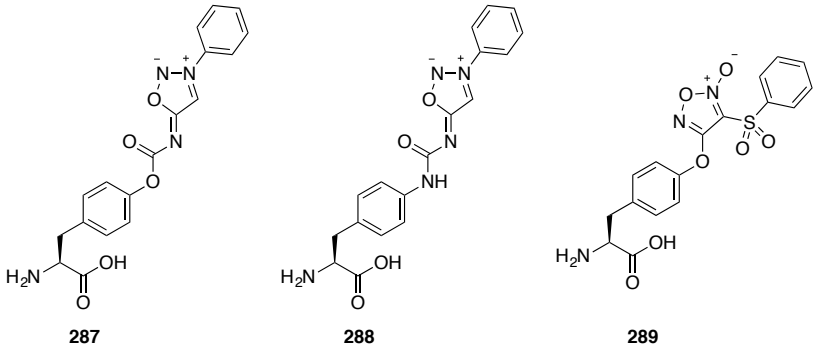
5.1 NITRIC OXIDE-DONATING AMINO ACIDS- RESULTS AND DISCUSSION

Four amino acid scaffolds were chosen, these were tyrosine **283**, 4-aminophenylalanine **284**, serine **285** and 2,3-diaminopropionic acid **286**.

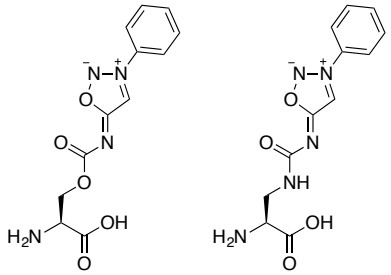


283 **284** **285** **286**

The NO donors chosen were the heterocyclic furoxan and sydnonimines. Following the earlier work, these were to be introduced using *bis*(phenylsulfonyl)furoxan **53** and *p*-nitrophenyl carbamate **218**. As such, the target amino acids are **287-291** respectively.

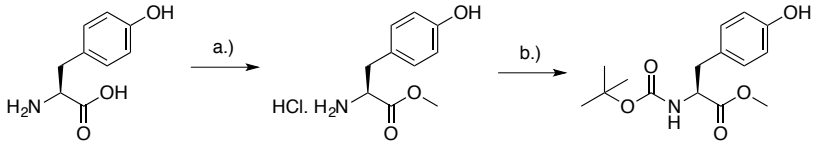


287 **288** **289**



290 **291**

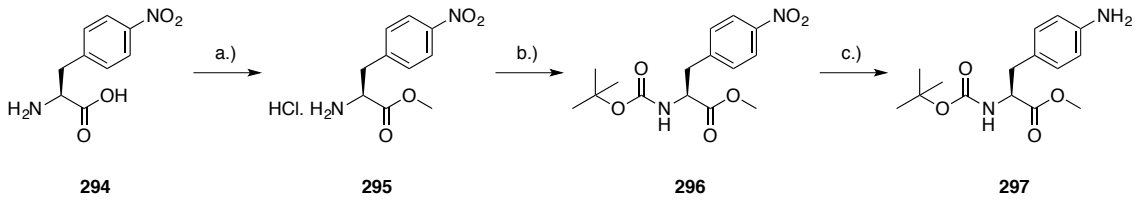
The amine and acid functionalities of tyrosine were protected based on literature procedures.[370,371] Tyrosine **283** was protected as its methyl ester **292** using MeOH/HCl in quantitative yield. Amine protection with di-*tert*-butyl dicarbonate (Boc_2O) provided the *tert*-butoxycarbonyl methyl ester **293** (Scheme 75).



283 **292** **293**

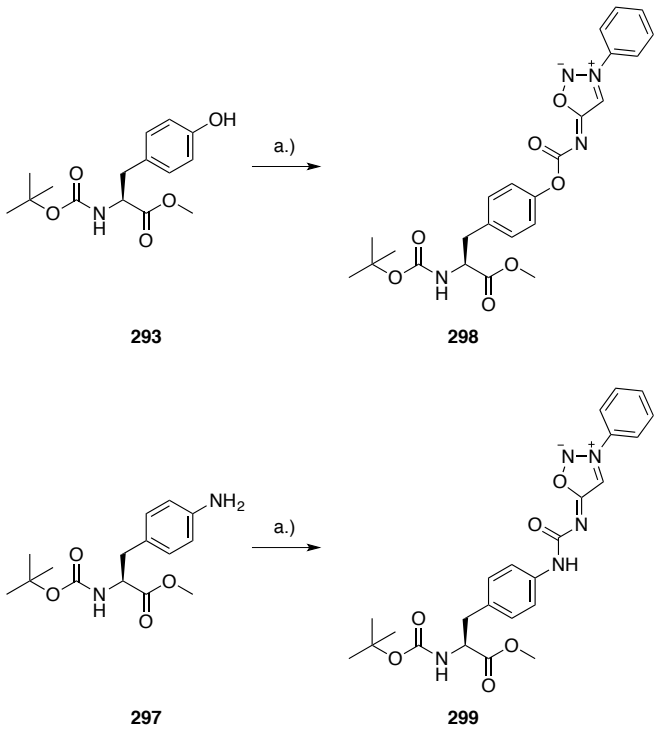
Scheme 75: *Reagents and conditions*: a.) AcCl, MeOH, reflux, 18 h, quant.; b.) Boc_2O, $NaHCO_3$, EtOH, r.t., 18 h, 80%.

4-Aminophenylalanine **284** was prepared from its nitro precursor **294**. A two step protection of 4-nitrophenylalanine **294** provided the *tert*-butoxycarbonyl methyl ester **296**. This was then efficiently reduced with palladium on carbon and ammonium formate to provide the aniline **297** (Scheme 76).



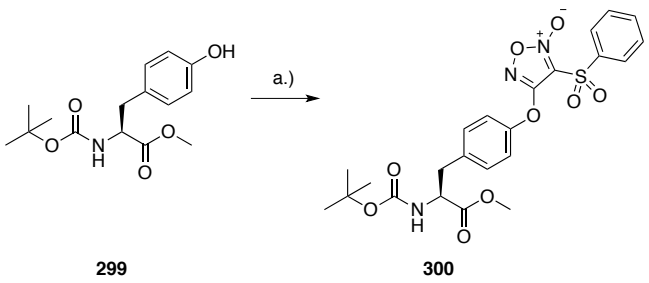
Scheme 76: *Reagents and conditions*: a.) $SOCl_2$, MeOH, 0 °C to r.t., 18 h, quant.; b.) Boc_2O, Et_3N, CH_2Cl_2, r.t., 18 h, quant.; c.) 10% Pd/C, ammonium formate, MeOH, r.t., 3 h, quant.

In order to prepare the protected amino acids with the sydnonimine heterocycle appended, **293** and **297** were treated with *p*-nitrophenyl carbamate **128** under the previously determined conditions. This furnished the desired carbamate **298** and urea **299** in excellent yields (Scheme 77).



Scheme 77: *Reagents and conditions*: a.) **218**, CH_3CN, reflux, 18 h, **298** = 89%, **299** = 85%.

Investigations were made to introduce the furoxan moiety into amino acids **293** and **297**. A modification of the conditions established by Fruttero was used.[372] The Fruttero conditions called for the use of 3 equiv. of alcohol, 2 equiv. of DBU and 1 equiv. of *bis*(phenylsulfonyl)furoxan **53**. However, the alcohols used were typically aliphatic alcohols, which could be removed on aqueous workup. Given that the tyrosine derivative **293** is not water soluble, it was chosen to use equimolar quantities of **293** and *bis*(phenylsulfonyl)furoxan **53**. In the event, treatment of phenol **293** with DBU, followed by addition of *bis*(phenylsulfonyl)furoxan **53** provided the desired furoxan ether **300** in 86% yield (Scheme 78).

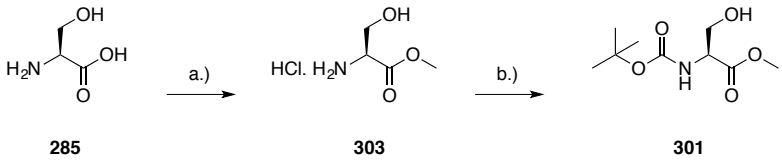


Scheme 78: *Reagents and conditions*: a.) **53**, DBU, CH_2Cl_2, r.t., 2 h, 86%.

Repeating these conditions with aniline **297** gave no reaction. In retrospect, there are no literature reports of *bis*(phenylsulfonyl)furoxan **53** reacting with nitrogen nucleophiles, and this reaction was unlikely to be successful.

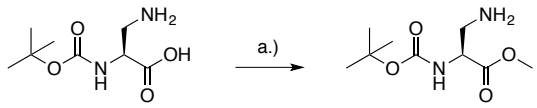
In addition to tyrosine-derived sydnonimines, the methodology was extended to serine **285** and 2,3-diaminopropionic acid **286**. The protected substrates **301** and **302** were prepared using standard literature methods.[373,374]

For serine **285**, protection as the methyl ester **303** with *in situ* generated HCl proceeded in quantitative yield, this was followed by *tert*-butoxycarbonyl protection with Boc_2O and triethylamine to give **301** (Scheme 79).



Scheme 79: *Reagents and conditions*: a.) $SOCl_2$, MeOH, 0 °C to r.t., 18 h, b.) Boc_2O, Et_3N, CH_3CN, r.t., 18 h, quant.

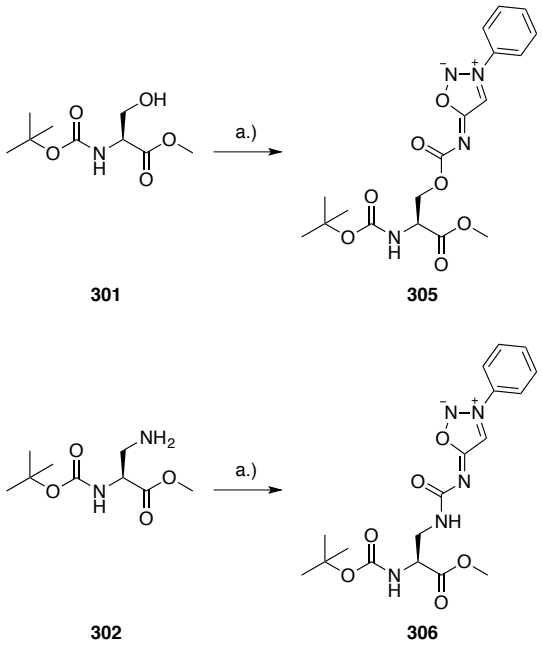
For 2,3-diaminopropionic acid **286**, commercially available (*S*)-2-[(*tert*-butoxycarbonyl)amino]-3-aminopropionic acid **304** was protected as its methyl ester **302** using TMS-diazomethane (Scheme 80).



304 **302**

Scheme 80: *Reagents and conditions*: a.) TMS-diazomethane, CH_2Cl_2, MeOH, r.t., 3 h, quant.

Substrates **301** and **302** were successfully acylated to give carbamate **305** and urea **306** in 60% and 55% yield, respectively (Scheme 81).

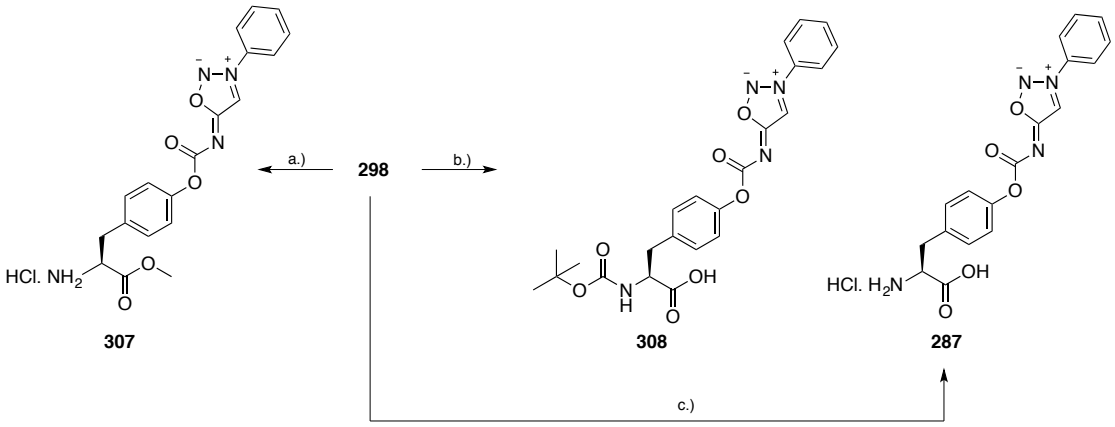


301 **305**

302 **306**

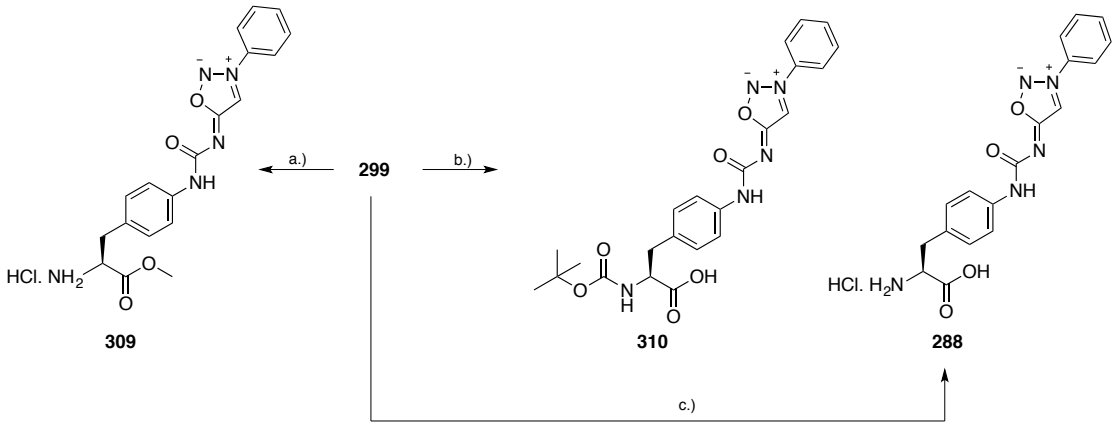
Scheme 81: *Reagents and conditions*: a.) **218**, CH_3CN, reflux, 18 h, **305** = 60%, **306** = 55%.

With five amino acid scaffolds in hand methods for achieving orthogonal deprotections were explored. Deprotections of **298** are summarised in Scheme 82.



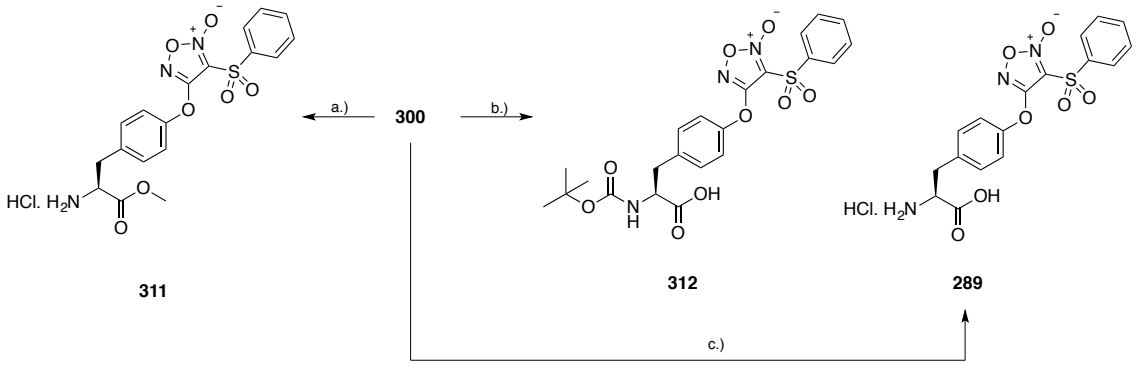
Scheme 82: *Reagents and conditions*: a.) 4 M HCl in dioxane, CH_2Cl_2, r.t., 1 h, quant.; b.) NaOH, THF:H_2O (1:1), r.t., 15 min, 95%; c.) i. NaOH, THF:H_2O (1:1), r.t., 15 min, ii. 4 M HCl in dioxane, CH_2Cl_2, r.t., 1 h, 77%.

tert-Butoxycarbonyl deprotection of **298** with HCl furnished the desired amine hydrochloride salt **307** in quantitative yield. Deprotection of the methyl ester was accomplished with NaOH in 1:1 THF:H_2O, providing carboxylic acid **308** after an acidic workup in good yield. A one-pot procedure combining these two steps, methyl ester deprotection and *tert*-butoxycarbonyl deprotection, furnished the free amino acid hydrochloride **287** in 77% overall yield. Application of these deprotection methods to urea **299**, furnished the amine hydrochloride **309**, acid **310** and amino acid **388** as anticipated (Scheme 83).



Scheme 83: *Reagents and conditions*: a.) 4 M HCl in dioxane, CH_2Cl_2, r.t., 1 h, quant.; b.) NaOH, THF:H_2O (1:1), r.t., 15 min, 88%; c.) i. NaOH, THF:H_2O (1:1), r.t., 15 min, ii. 4 M HCl in dioxane, CH_2Cl_2, r.t., 1 h, 75%.

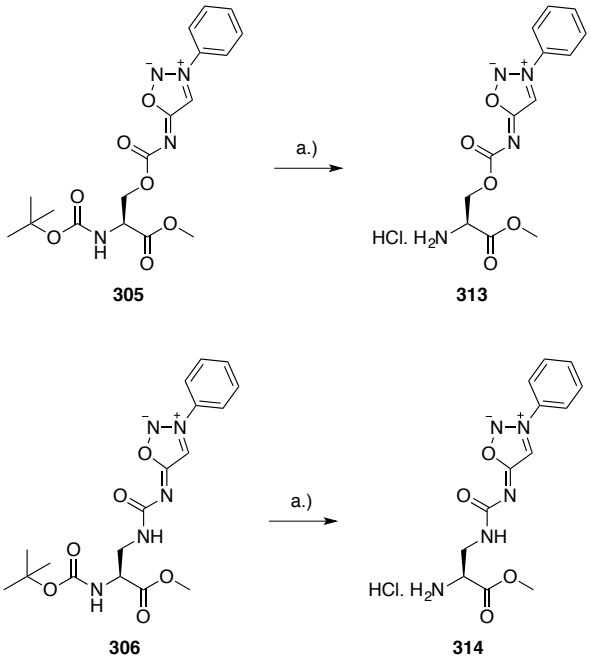
The ether **300** could also be selectively deprotected to the amine hydrochloride **311**, carboxylic acid **312**, and amino acid **289** under the similar reaction conditions (Scheme 84).



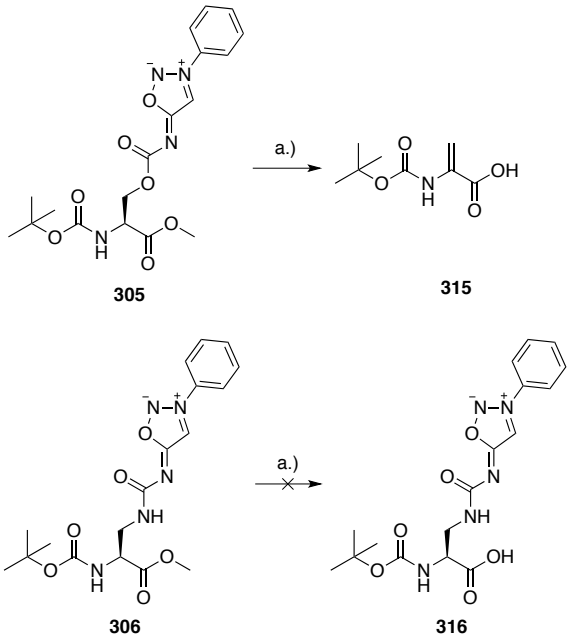
Scheme 84: *Reagents and conditions*: a.) 4 M HCl in dioxane, CH_2Cl_2, r.t., 1 h, quant.; b.) NaOH, THF:H_2O (1:1), r.t., 15 min, 90%; c.) i. NaOH, THF:H_2O (1:1), r.t., 15 min, ii. 4 M HCl in dioxane, CH_2Cl_2, r.t., 1 h, 80%.

tert-Butoxycarbonyl deprotections of **305** and **306** under acidic conditions gave the desired amine hydrochlorides **313** and **314** (Scheme 85) in a straight forward manner.

However, ester hydrolysis of **305** with NaOH generated the dehydroalanine carboxylic acid **315**. A similar reaction with **306** did not give any recoverable material (Scheme 85).

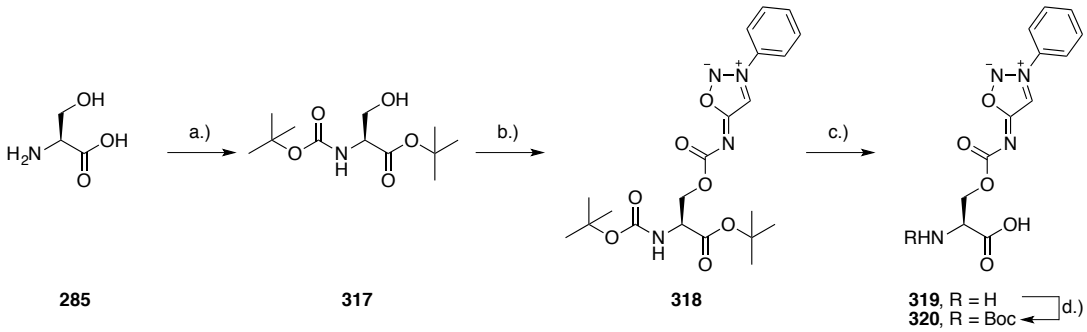


Scheme 85: *Reagents and conditions*: a.) 4 M HCl in dioxane, CH_2Cl_2, r.t. 1 h, **313** = **314** = quant.



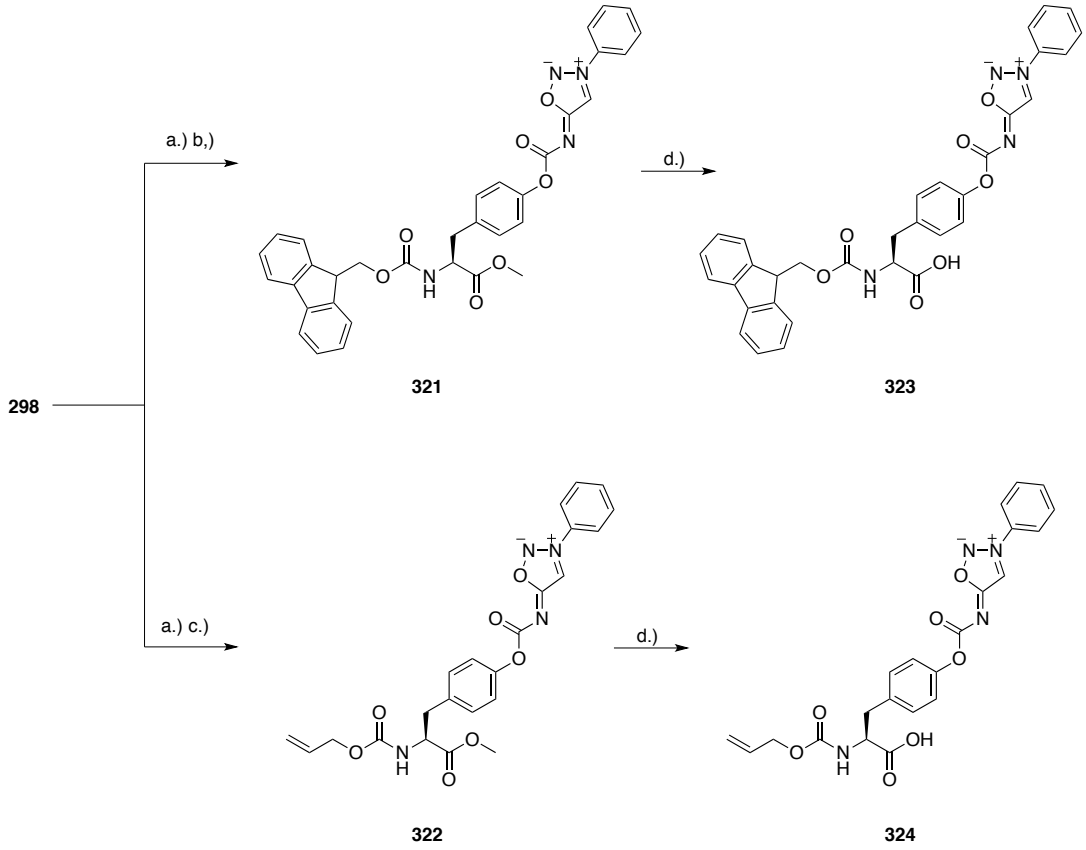
Scheme 86: *Reagents and conditions*: a.) NaOH, THF:H_2O, r.t., 15 min, **315** = yield not determined, **316** = no product isolated.

To access the desired free carboxylic acid derivative of **305**, serine **285** was protected as its *tert*-butoxycarbonyl *tert*-butyl ester **317** (Scheme 87). Acylation with *p*-nitrophenyl carbamate **218** provided sydnonimine **318** in 60% yield and then global deprotection with trifluoroacetic acid furnished the amino acid trifluoroacetate **319**. *N*-Protection with Boc_2O then gave the desired free carboxylic acid **320** (Scheme 87).



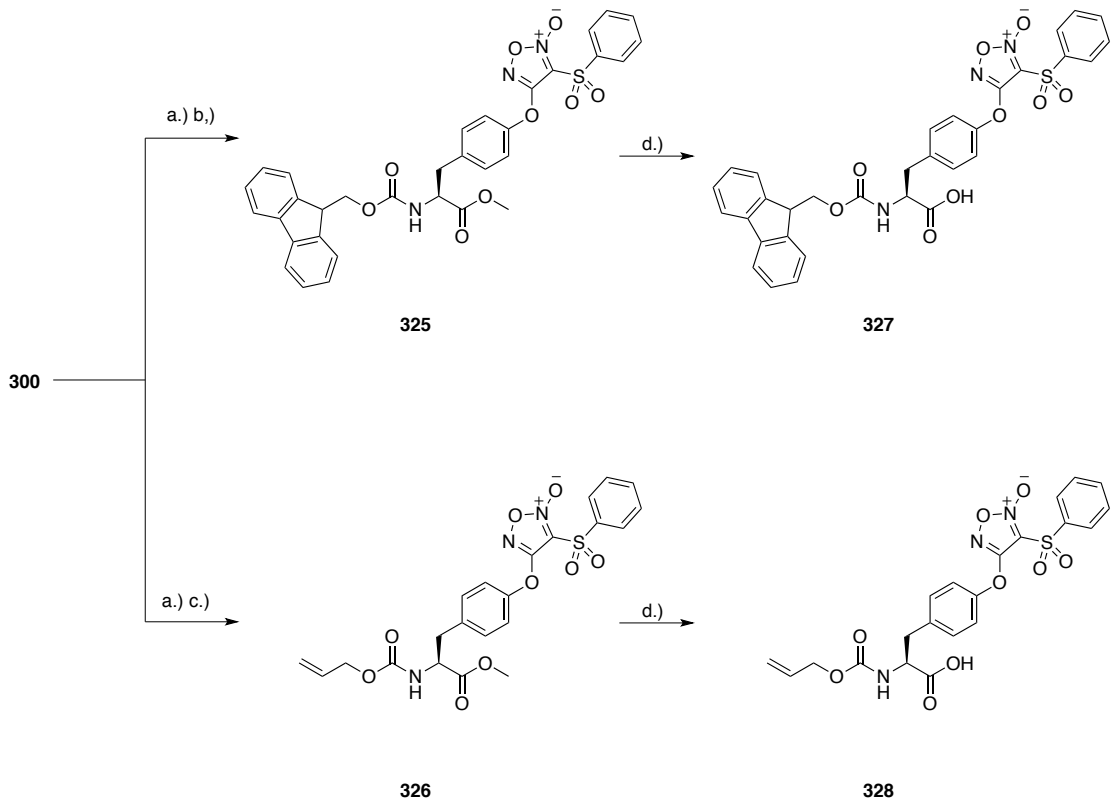
Scheme 87: *Reagents and conditions*: a.) i. Boc_2O, NaOH, dioxane, 18 h; ii. DMF-DBA, toluene, reflux, 18 h, 65%; b.) **218**, CH_3CN, reflux, 18 h, 60%; c.) TFA, CH_2Cl_2, r.t., 16 h, quant.; d.) Boc_2O, $NaHCO_3$, H_2O:dioxane (1:1), r.t., 16 h, 72%.

While the *tert*-butoxycarbonyl group is a robust protecting group for amines, it is not always compatible with further synthetic chemistry. Its sensitivity towards acid precludes its use with other acid sensitive protecting groups such 2,2,4,6,7-pentamethyl-dihydrobenzofuran-5-sulfonyl (Pbf) and *ter*t-butyl ether/esters, where selective protection is required. To allow for the amino acid to be used in further chemistry, **298** and **300** were orthoganally with two different groups, Fmoc and Alloc. Deprotection of **298** with HCl furnished the amine hydrochloride **307** as previously described (Scheme 82). The amine hydrochloride salt **307** was then treated with Fmoc-Cl or Alloc-Cl in THF to re-protect the free amine providing the Fmoc **321** and the Alloc **322** amino acid (Scheme 88). These two amino acids were deprotected with NaOH to provide the free carboxylic acids **323** and **324**, respectively (Scheme 88).



Scheme 88: *Reagents and conditions*: a.) 4 M HCl in dioxane, CH_2Cl_2, r.t., 1 h, quant.; b.) Fmoc-Cl, Et_3N, THF, 0 °C to r.t., 1 h, 90%; c.) Alloc-Cl, Et_3N, THF, 0 °C to r.t., 1 h, 85%; d.) NaOH, THF:H_2O, r.t., 15 min, **323** = 80%, **324** = 80%.

The same conditions were applied to amino acid **300** to furnish Fmoc **325** and Alloc **326** amino acid, along with the free carboxylic acids **327** and **328** after methyl ester deprotection (Scheme 89).



Scheme 89: *Reagents and conditions*: a.) 4 M HCl in dioxane, CH_2Cl_2, r.t., 1 h, quant.; b.) Fmoc-Cl, Et_3N, THF, 0 °C to r.t., 1 h, 90%; c.) Alloc-Cl, Et_3N, THF, 0 °C to r.t., 1 h, 81%; d.) NaOH, THF:H_2O, r.t., 15 min, **327** = 80%, **328** = 77%.

5.1.1 NITRIC OXIDE RELEASE

The Griess test was used to measure the NO release from amino acids **287** and **289**, as they are the precursor building blocks for further chemistry. The Griess test is a colourimetric assay for nitrite, one of the oxidation products of NO (see Chapter One for detailed description).[188] While the measurement of nitrite is not a direct measure of NO, it is a reliable proxy measure due to the rapid oxidation of NO. For furoxans, it has previously been reported that the formation of nitrite (NO_2^-), is a reliable measure of the NO release, in the presence of excess thiol.[139] Equimolar production of superoxide ($O_2^{\bullet}$), along with NO is reported, for sydnonimines.[140] To this affect, the Griess assay for NO_2^- was used to determine the production of NO from amino acids **287** and **289**, and as a control, L-tyrosine **283**. Amino acids **287** and **289** (1 mmol, [final], 400 μM) in

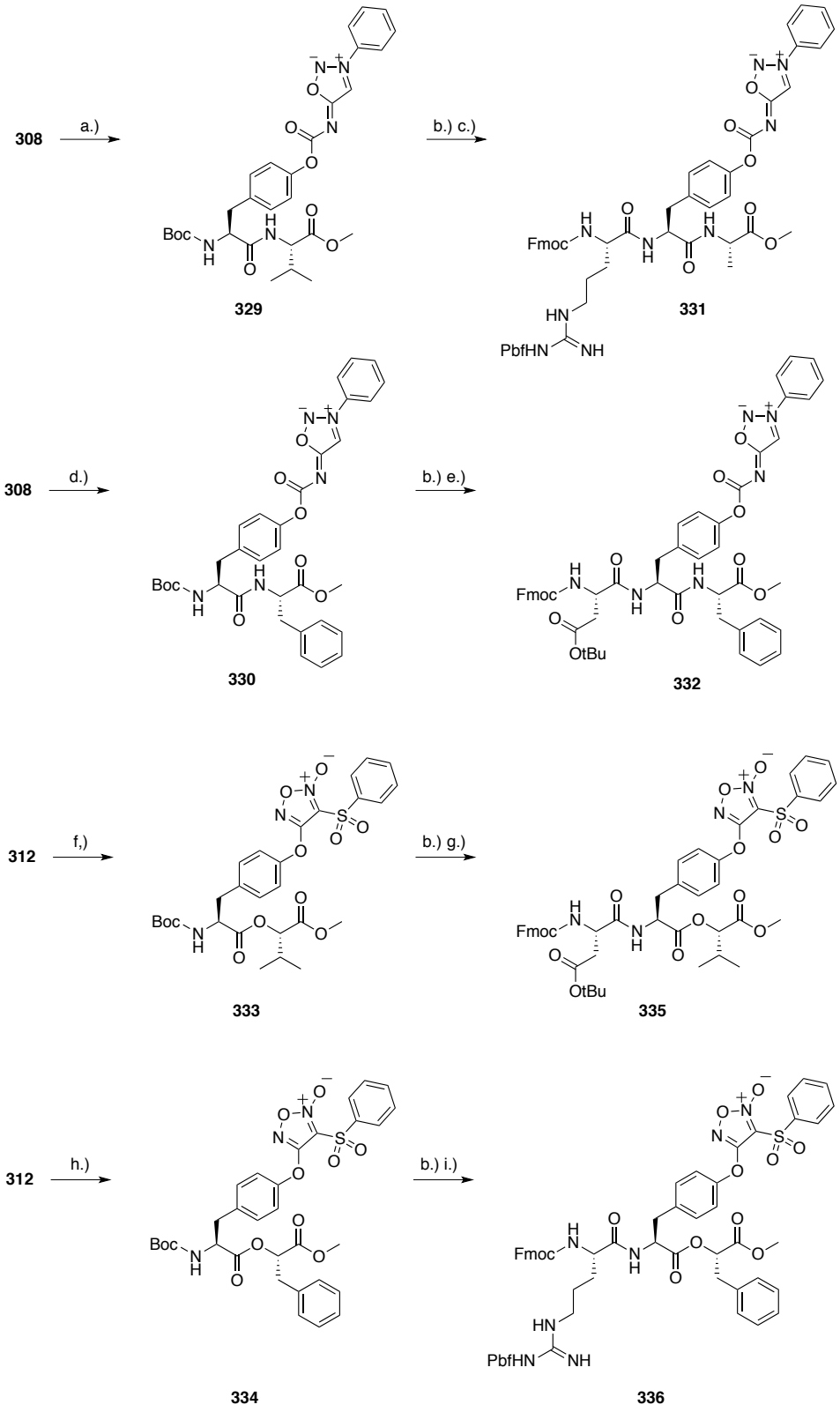
a solution of DMSO: H_2O (2:8) were incubated with a glutathione (GT) buffer containing superoxide dismutase (SOD) ([GT] 6 mmol, final [GST] 600 μM, pH 7.6, [SOD] 4557 U mL^{-1}). Amino acids **287** and **289** showed an NO_2^- release of 30.4 ± 3.9 μmol and 44.8 ± 3.2 μmol, respectively. (Table 20) The amount of NO required to induce a cellular response is at the picomolar to nanomolar range. Control incubations performed under identical conditions in the absence of GT and SOD did not give significant NO production. This is in agreement with the reported release of NO from furoxans and sydnonimines in aqueous conditions,[139,140] No measureable NO production was observed from tyrosine **283**.

Compound	NO_2^- production (μmol) (GT buffer)	NO_2^- production as % (GST buffer)	NO_2^- production (μmol) (pH 7.6 buffer)	NO_2^- production as % (pH 7.6 buffer)
287	30.4 (± 3.9)	7.5	3.2 (± 1.1)	<1%
289	44.8 (± 3.2)	11.2	5.2 (± 5.5)	1.3 %
293	0.0 (± 0.1)	0	0.0 (± 0.1)	0

Table 20: NO release from amino acids **287** and **289.**

To demonstrate the synthetic utility of these amino acids, **287** and **289** were incorporated into tripeptide sequences (Scheme 90).

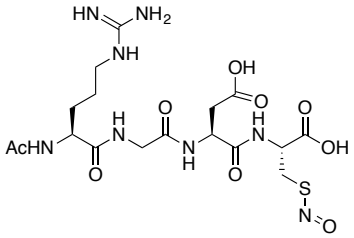
Amide formation between **308** with L-Val-OMe and L-Phe-OMe with HATU in DMF provided the dipeptides **329** and **330** in >99:1 *d.r.* and in 91% and 95% yields, respectively (**Scheme 90**). Deprotection with HCl provided the hydrochloride salt, which was not characterised, but immediately to couple to Fmoc-Arg(Pbf)-OH and Fmoc-Asp(OtBu)-OH to provide the tripeptides **331** and **332** (**Scheme 90**). The conditions were applicable to amino acid **312** to furnish dipeptides **333** and **334**, and tripeptides **335** and **336** (Scheme 90).



Scheme 90: *Reagents and conditions*: a.) L-Val-OMe, HATU, $(iPr)_2EtN$, DMF, 0 °C to r.t., 16 h, **329** = 91%; b.) 4 M HCl in dioxane, CH_2Cl_2, 1 h; c.) Fmoc-L-Arg(Pbf)-OH, HATU, $(iPr)_2EtN$, DMF, 0 °C to r.t., 16 h, **331** = 88%; d L-Phe-OMe, HATU, $(iPr)_2EtN$, DMF, 0 °C to r.t., 16 h, **330** = 95%; e.) Fmoc-L-Asp(OtBu)-OH, HATU, $(iPr)_2EtN$, DMF, 0 °C to r.t., 16 h, **332** = 85%; f.) L-

Val-OMe, HATU, $(iPr)_2EtN$, DMF, 0 °C to r.t., 16 h, **333** = 95%; g.) Fmoc-L-Asp(OtBu)-OH, HATU, $(iPr)_2EtN$, DMF, 0 °C to r.t., 16 h, **335** = 80%; h.) L-Phe-OMe, HATU, $(iPr)_2EtN$, DMF, 0 °C to r.t., 16 h, **334** = 95%; i.) Fmoc-L-Arg(Pbf)-OH, HATU, $(iPr)_2EtN$, DMF, 0 °C to r.t., 16 h, **336** = 88%.

With the development of protecting group strategies for the amino acid series, attention turned to their integration into biologically relevant peptides. The RGD peptide sequence was identified as a suitable framework. NO-RGD peptides could clearly target nitric oxide delivery to the surface of cancer cells. Given the previously reported findings that the hypoxic response is reversed in prostate cancer cells upon treatment with nitric oxide NSAIDs,[298] an NO-RGD peptide may allow for a more targeted delivery of NO to a cell, rather than from a compound such as NCX-1102 **111**, not a prostate specific compound. NO-RGD peptides also have the potential for use in cardiovascular disease. It has been reported that the tetrapeptide RGD peptide **337,** containing an *S*-nitrosothiol-derived cysteine residue, was a more effective antithrombotic agent than the RGD sequence alone.[375] RGD-SNO peptide **337** is also a vasodilatory peptide, due to the release of NO. RGD alone did not cause vasodilation.[375]



337

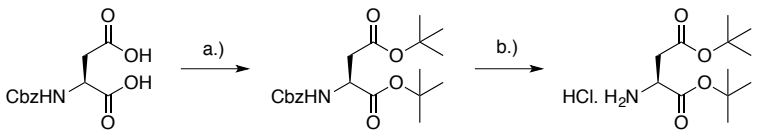
Two types of NO-RGD peptides were designed for synthesis. The first are linear peptides: containing the RGD sequence with an NO-release functionality. The second, a cyclic RGD pentapeptide containing amino acid **287**. Cyclic RGD peptides

conformationally lock the RGD sequence and in general have a greater affinity for integrin binding and increased *in vivo* stability.

5.2 NITRIC OXIDE-DONATING RGD PEPTIDES- RESULTS AND DISCUSSION

A solution phase Fmoc/tBu peptide coupling strategy was chosen for the preparation of the linear RGD peptides. This strategy would assemble the RGD sequence first, followed by coupling of the desired carboxylic acid. Global deprotection would then provide the peptide.

The desired RGD sequence was prepared based on the procedure reported by Welsh and Smith,[376] starting from di-*tert* butyl ester protected aspartic acid **338**. This was carried out in two steps from commercially available *N*-Cbz-aspartic acid **339** (Scheme 91).



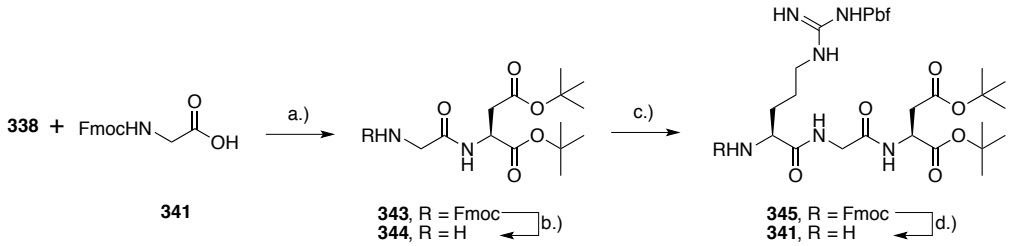
339 **340** **338**

Scheme 91: *Reagents and conditions*: a.) *tert*-Butyl acetate, $BF_3(OEt_2)$, r.t., 18 h, 60%; b.) i. NH_4CO_2H, 10% Pd/C, THF/MeOH (1:1), r.t., 18 h, ii.) sat. HCl in EtOAc, 1 h, -20 °C, 80%.

Protection of the free carboxylic acid groups of **339** was accomplished using *tert*-butyl acetate and boron trifluoride (Scheme 91).[377] Workup using aqueous NaOH allowed for the unreacted starting material and mono-protected acid to be extracted into the aqueous layer, such that the isolated fully protected **340** required no further purification. Deprotection of the Cbz group was accomplished by transfer hydrogenation with ammonium formate and palladium on carbon.[377] The free base was converted to its hydrochloride salt **338** by the addition of a saturated solution of HCl in ethyl acetate to a

solution of the amine in ethyl acetate. The overall yield of the two steps was 48%, and the reaction could be carried out on a 10 g scale.

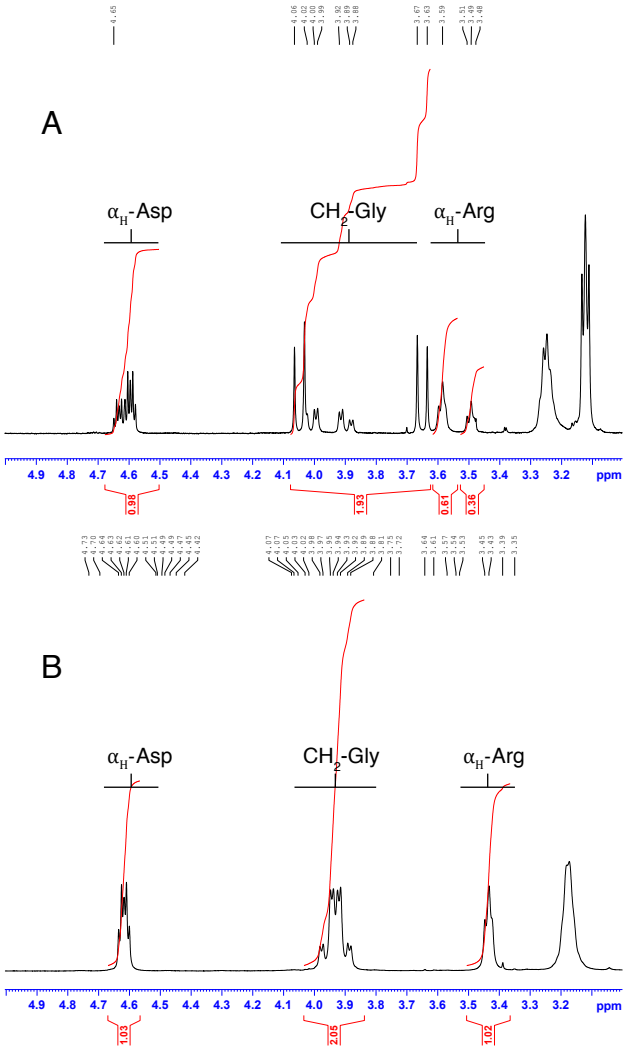
The synthesis of NH_2-RGD **341** was undertaken as reported by Welsh and Smith (Scheme 92).[376]



Scheme 92: *Reagents and conditions*: a.) T3P®, $(iPr)_2EtN$, CH_2Cl_2, 0 °C, 16 h, 95%; b.) piperidine, CH_2Cl_2, 0 °C to r.t., 2 h, 87%; c.) T3P®, $(iPr)_2EtN$, CH_2Cl_2, 0 °C, 16 h, 90%; d.) piperidine, DMF, 0 °C to r.t., 0.5 h, 85%.

Peptide coupling between the prepared di-*tert* butyl aspartic acid **338** and Fmoc-Gly-OH **341** using T3P® furnished dipeptide **343** in 95% yield.[376] The observed optical rotation of this material was +23.0 (c = 1.0, $CHCl_3$), in accordance with the reported value +22.9 (c = 1.0, $CHCl_3$).[376] Deprotection of the Fmoc group was carried out using piperidine to give amine **344**.[376] Homologation of amine **344** with commercially available Fmoc-Arg(Pbf)-OH furnished tripeptide **345**.[376] The crude material was a 99:1 mixture of diastereoisomers based on ^{1}H NMR; and it was possible to separate the undesired diastereoisomer by chromatography and isolate the desired tripeptide **345** in 85% yield.

Fmoc deprotection of **345** following the literature method[376] resulted in a 70% racemisation of the arginine stereogenic centre **(**Figure 17**)**.



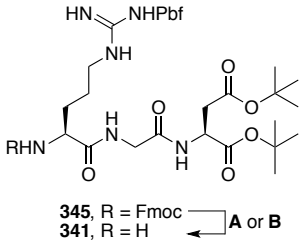
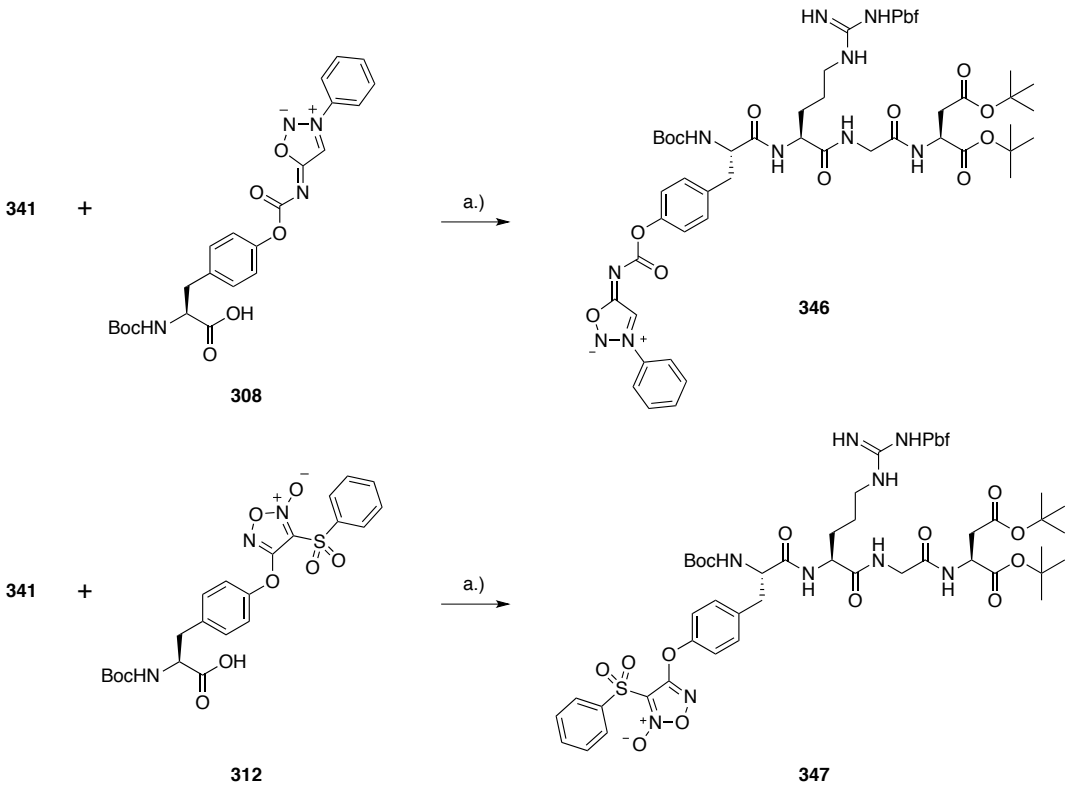


Figure 17: ^{1}H NMR (500 MHz, $CDCl_3$) comparison of Fmoc deprotection of **345**.

A = 20% piperidine in CH_2Cl_2,

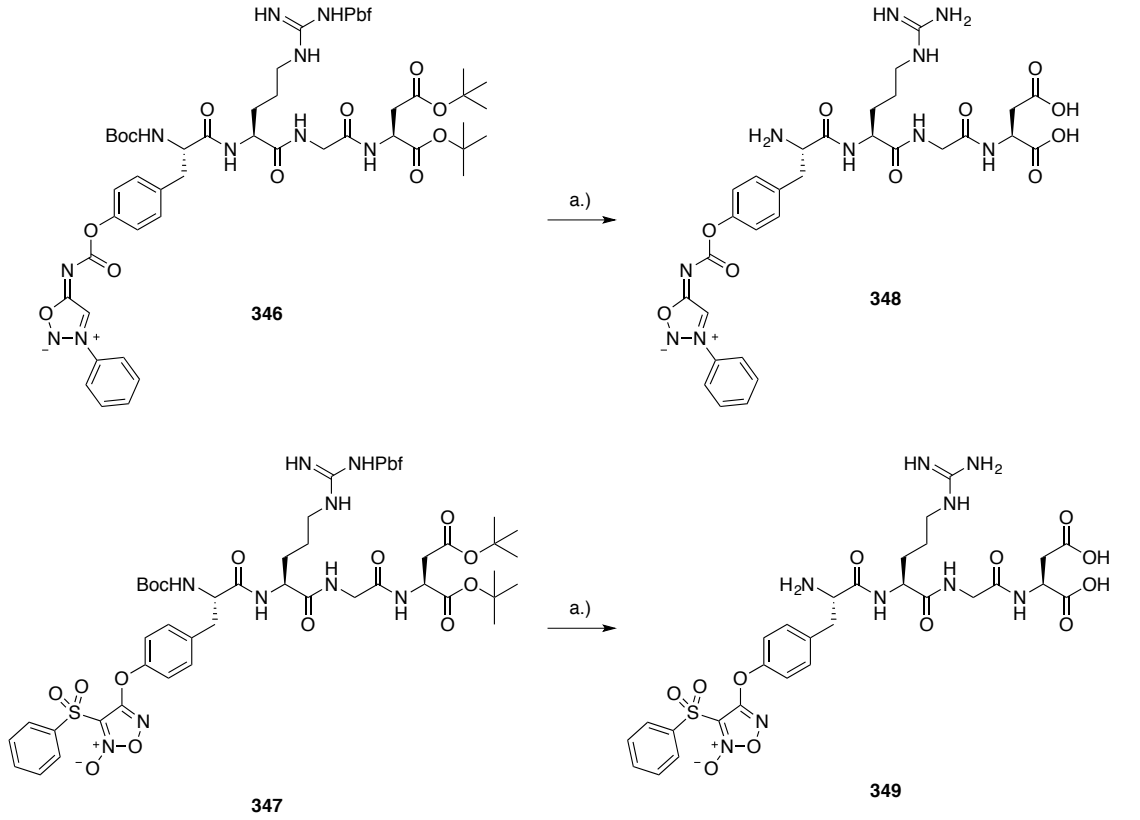
B = 10% piperidine in DMF.

Changing the deprotection solution from 20% piperidine in dichloromethane to 10% piperidine in DMF arrested the racemisation and generated diasteromerically pure **341**. With amine **341** in hand it was coupled to the Boc-protected amino acids **308** and **312** using HATU as the coupling reagent, to generate tetrapeptides **346** and **347** in excellent yield and as single diastereoisomers after column chromatography (Scheme 93).



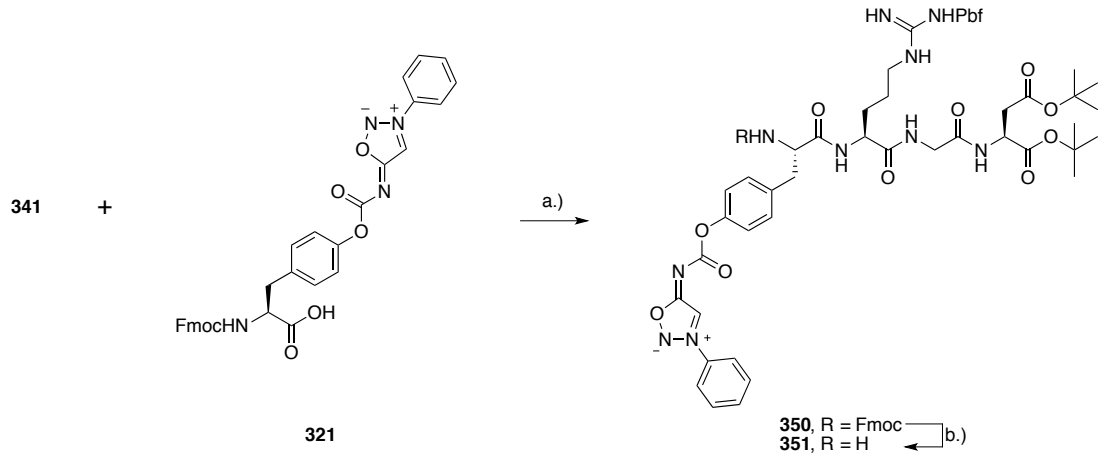
Scheme 93: *Reagents and conditions*: a.) HATU, DMF, $(iPr)_2EtN$, 0 °C to r.t., 18 h, **346** = 70%, **347** = 69%.

The acid labile Boc, Pbf and *tert*-butyl esters were then globally deprotected by treatment with a cocktail of TFA, triisopropylsilane (TIPS) and water (95:2.5:2.5). This furnished the tetrapeptides **348** and **349** as their trifluoroacetate salts after lyophilisation.



Scheme 94: *Reagents and conditions*: a.) TFA:TIPS:H_2O, r.t., 2 h, **348** = 92%, **349** = 90%.

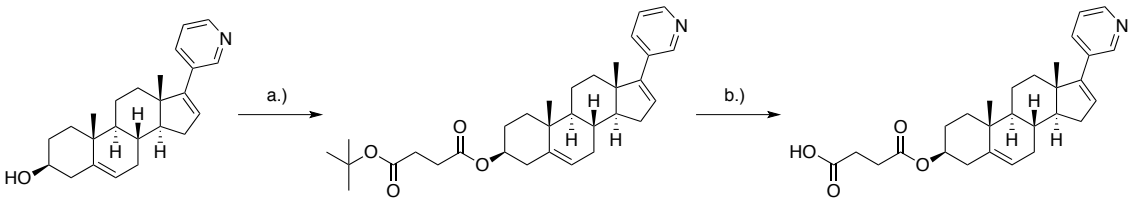
By using Fmoc-protected amino acid **321** the tetrapeptide **350** could also be accessed under the same coupling conditions (Scheme 95).



Scheme 95: *Reagents and conditions*: a.) HATU, DMF, $(iPr)_2EtN$, 0 °C to r.t., 18 h, 76%; b.) piperidine, DMF, 0 °C to r.t., 0.5 h, 90%,

The use of the Fmoc allowed for selective deprotection at the *N*-terminus furnishing the free amine **351** for use in further chemistry (Scheme 95). To demonstrate this, the NO-RGD peptide **351** was linked to abiraterone **106** *via* a succinate linker. This molecule combines the bioactive properties of abiraterone **106**, an NO release unit, and an RGD targeting sequence.

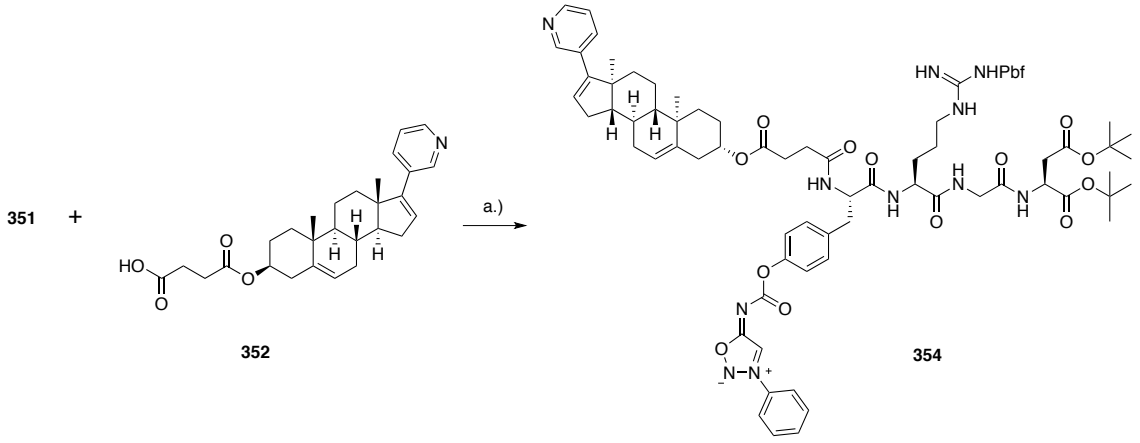
Abiraterone hemisuccinate **352** was prepared in two steps using previously prepared *tert*-butyl hemisuccinate **264** (Scheme 96).



Scheme 96: *Reagents and conditions*: a.) **264**, EDCI.HCl, DMAP, CH_2Cl_2, r.t., 16 h, 95%; b.) TFA, CH_2Cl_2, r.t., 16 h, quant.

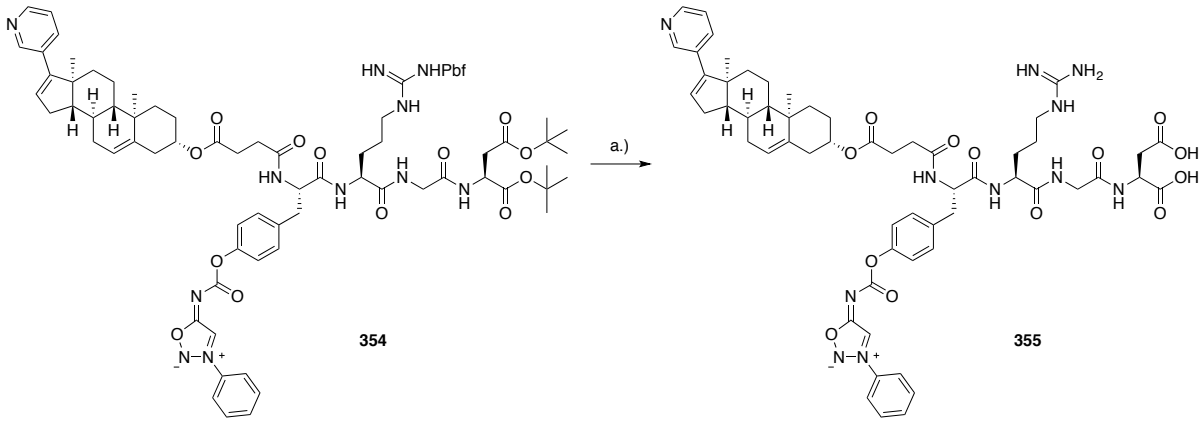
tert-Butyl ester **353** was prepared in 95% yield from abiraterone **106** and *tert*-butyl hemisucinate with EDCI.HCl and DMAP (Scheme 96). Deprotection of **353** with trifluoroacetic acid furnished hemisuccinate **352** in quantitative yield (Scheme 96). Attempts to prepare **352** directly from abiraterone **106** were investigated, however the reaction with succinic anhydride **263** was slow, and purification of the product proved difficult.

With hemisuccinate **352** in hand, this was coupled to amine **351** to provide the RGD-abiraterone conjugate **354** (Scheme 97).



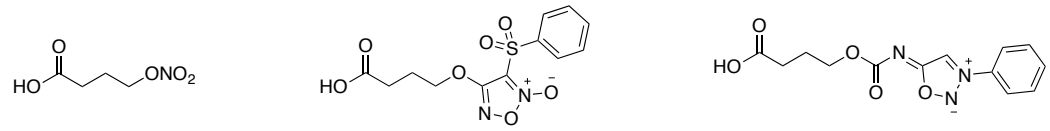
Scheme 97: *Reagents and conditions*: a.) HATU, DMF, $(iPr)_2EtN$, 0 °C to r.t., 18 h, 83%.

The ^{1}H and ^{13}C resonances of conjugate **354** could be fully assigned using 2D COSY, HSQC and HMBC analysis. Deprotection of the conjugate with the TFA cocktail, followed by trituration with diethyl ether, and then lyophilisation, gave the final conjugate **355** (Scheme 98).

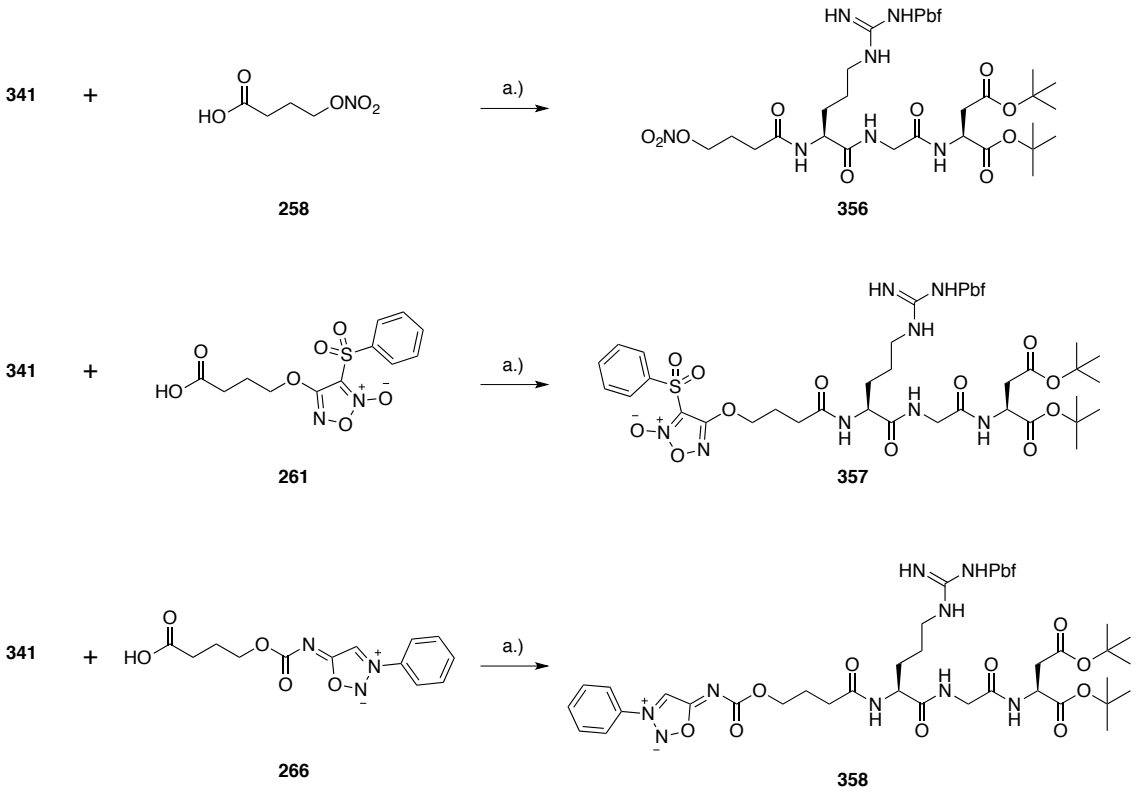


Scheme 98: *Reagents and conditions*: a.) TFA:TIPS:H_2O, r.t., 2 h, 75**%,**

RGD amine **341** was also coupled with carboxylic acids **258**, **261** and **266**. These were prepared earlier in Chapter Three. Peptide coupling using HATU as the activation reagent furnished the peptides **356-357** in good yields (Scheme 99). Finally, subsequent deprotection and lyophilisation furnished peptides **359-361** (Scheme 100).



258 **261** **266**

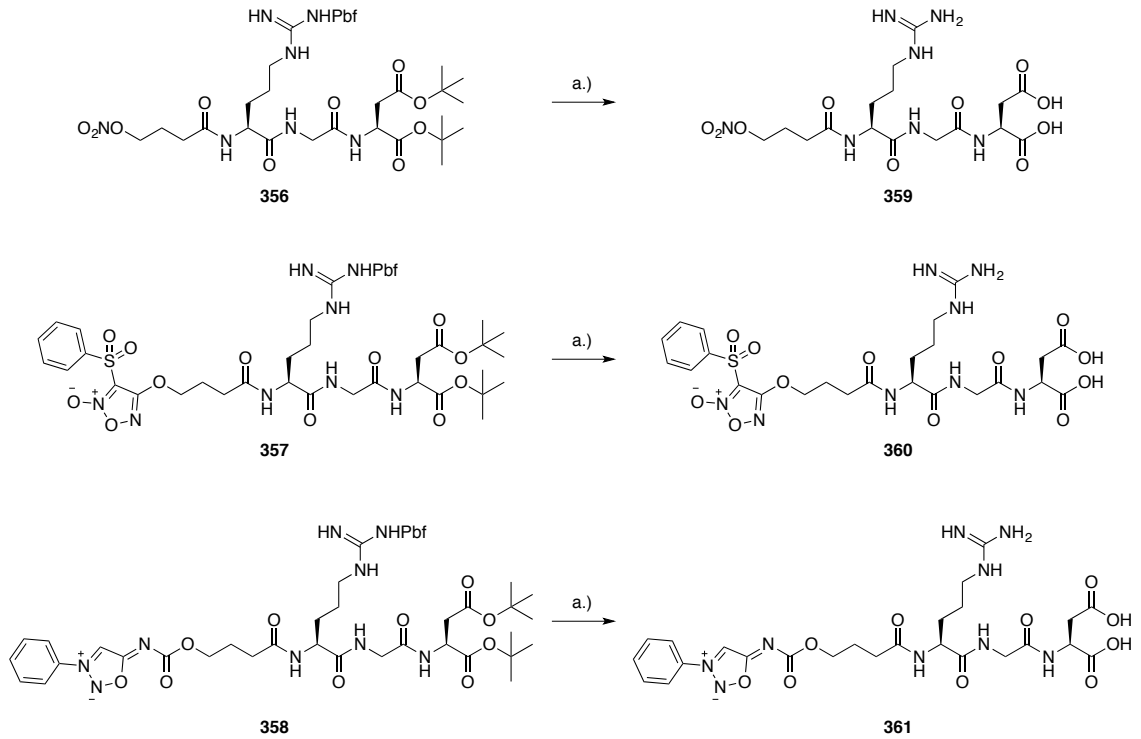


341 + **258** → **356**

341 + **261** → **357**

341 + **266** → **358**

Scheme 99: *Reagents and conditions*: a.) HATU, DMF, $(iPr)_2EtN$, 0 °C to r.t., 18 h, **358** = 65%, **359** = 76%, **360** = 80%.



356 **359** **357** **360** **358** **361**

Scheme 100: *Reagents and conditions*: a.) TFA:TIPS:H_2O, r.t., 2 h, **359** = 85%, **360** = 90%, **361** = 88%.

5.2.1 NITRIC OXIDE RELEASE

The Griess test was used to determine the level of NO release from the NO-RGD peptides.[188] Solutions of RGD peptides **348**, **349**, **355**, **359-361** (1 mmol, [final], 400 μM) in DMSO: H_2O (1:9) were incubated with a GT buffer containing SOD ([GT] 6 mmol, final [GT] 600 μM, pH 7.6, [SOD] 4557 U mL^{-1}). RGD was used as a control. The data are summarized in Table 21.

Compound	NO_2^- production (µmol) (GT buffer)	NO_2^- production as % (GST buffer)	NO_2^- production (µmol) (pH 7.6 buffer)	NO_2^- production as % (pH 7.6 buffer)
359	32.2 ± 2.5	8.1	2.1 ± 0.4	<1.0
360	41.4 ± 1.1	10.4	1.6 ± 1.1	<1.0
361	29.6 ± 6.6	7.4	3.1 ± 2.3	<1.0
348	40.8 ± 2.7	10.2	4.0 ± 1.6	1.0
349	52.5 ± 5.4	13.1	1.0 ± 0.7	<1.0
355	31.6 ± 4.8	7.9	2.4 ± 1.1	<1.0
270	2.6 ± 0.6	<1.0	2.2 ± 0.7	<1.0

Table 21: NO release from RGD peptides. Data are the averages of triplicate experiments

All of the prepared NO-RGD peptides demonstrated GT dependent NO release in the Griess assay. The RGD sequence **270** alone did not show any NO-release in GT or pH 7.6 buffer. The highest levels of NO release were observed from the furoxan RGD peptides **349** and **360**. The levels of NO release were 41.4 ± 1.1 µmol and 52.5 ± 5.1 µmol (10.4 and 13.1%) respectively. Sydnonimine RGD peptide **348** had a similar level of NO release (40.8 ± 2.7 µmol, 10.2 %) to furoxan RGD peptides **349** and **360**. Nitrate ester **359** and abiraterone conjugate **355** had comparable levels of NO release (32.2 ± 2.5 µmol and 31.6 ± 4.8 µmol)

5.2.2 $\alpha_v\beta_3$ INTEGRIN AFFINITY EVALUATION

The RGD peptide conjugates were submitted to integrin binding assays to measure their affinity for the $\alpha_v\beta_3$ integrin. These assays were conducted by Dr Ian Fleming at the University of Aberdeen, Biomedical Imaging Centre.

The competitive affinity of RGD peptides **348**, **349**, **355**, **359-361** were calculated based on their affinity towards an immobilized $\alpha_v\beta_3$ integrin receptor and a biotinylated c[RGDfK]-$(PEG)_2$. Commercially available RGD **270** and GRGDSPK were used as reference sequences. IC_{50} values were calculated for each peptide, and were normalized

with respect to the IC_{50} of RGD to allow comparisons between experiments. The results are summarised in Table 22.

Compound	IC_{50} / μm	*Q*
RGD	8.5 ± 3.1	1
GRGDSPK	6.4 ± 1.3	0.75
359	5.6 ± 0.5	0.66
360	4.7 ± 0.7	0.56
361	6.2 ± 0.2	0.73
348	6.0 ± 0.3	0.70
349	5.8 ± 1.6	0.68
355	107.6 ± 45.7	12.6

Table 22: Inhibition of biotinylated c[RGDfK]-$(PEG)_2$ binding to immobilized $\alpha_v\beta_3$ integrin. Data are the averages of triplicate experiments. *Q* = normalized activity as ratio IC_{50}(peptide)/IC_{50}(RGD)

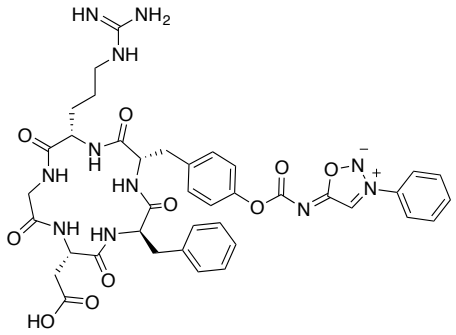
The highest affinity (4.8 μM) was observed for furoxan RGD peptide **360**. This was nearly two-fold higher affinity than RGD **360**, and nearly 1.5 fold higher affinity than GRGDSPK. RGD peptides **359**, **361**, **348**, **349** all displayed greater affinity for $\alpha_v\beta_3$ compared to RGD, and they had a comparable activity to GRGDSPK.

Abiraterone conjugate **355**, displayed an integrin binding affinity of 107.6 μM, an approximately 13-fold decrease in affinity compared to RGD. The lower affinity of **355** might be the result of the increased structural complexity of the molecule, allowing fewer degrees of freedom of rotation while maintaining integrin binding.

The integrin binding studies suggest that the addition of a single amino acid residue, or a heterocycle appended chain onto the *N*-terminus of the peptide has little affect on the affinity of the peptide to $\alpha_v\beta_3$. However, installation of a large group on the N-terminus as shown with peptide **355** results in a decreased affinity towards the $\alpha_v\beta_3$ integrin when a linear chain is used.

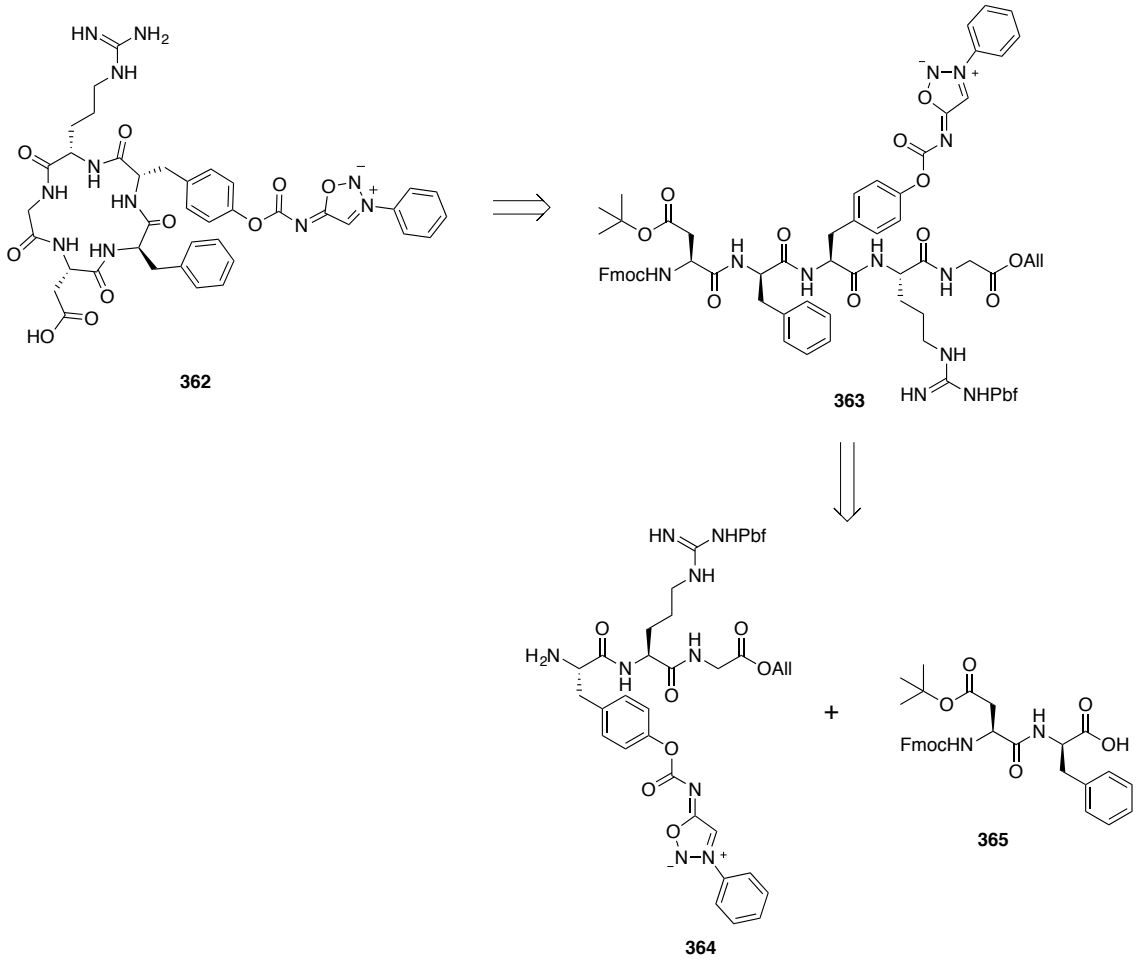
5.3 STUDIES TOWARDS THE SYNTHESIS OF A NITRIC OXIDE-DONATING CYCLIC RGD PEPTIDE

Cyclic RGD peptides generally have an improved integrin binding affinity compared to linear RGD sequences. Macrolactamisation of the RDG sequence entropically favours RGD binding by pre-organisation of the recognition sequence. In addition, cyclic RGD's have improved metabolic stability and these are attractive targets. Cyclic RGD **362** was proposed as a synthetic target.



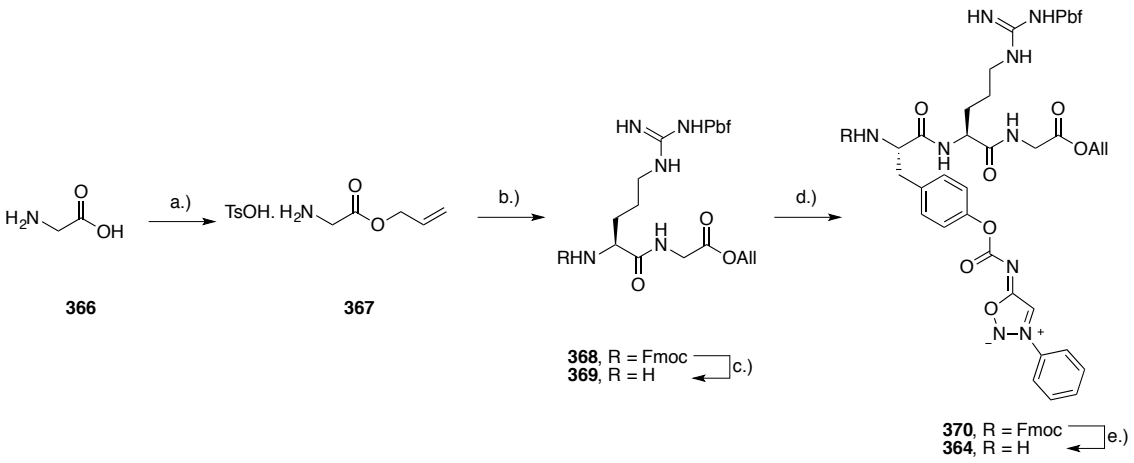
362

The retrosynthetic strategy towards peptide **362** is based on the macrolactamisation of acyclic peptide **373**. This can be prepared in a convergent approach from two fragments. Fragment A is the tripeptide **374** with a free amine functionality; Fragment B is the carboxylic acid **375** (Scheme 101).



Scheme 101: Retrosynthetic analysis of cRGD **362**.

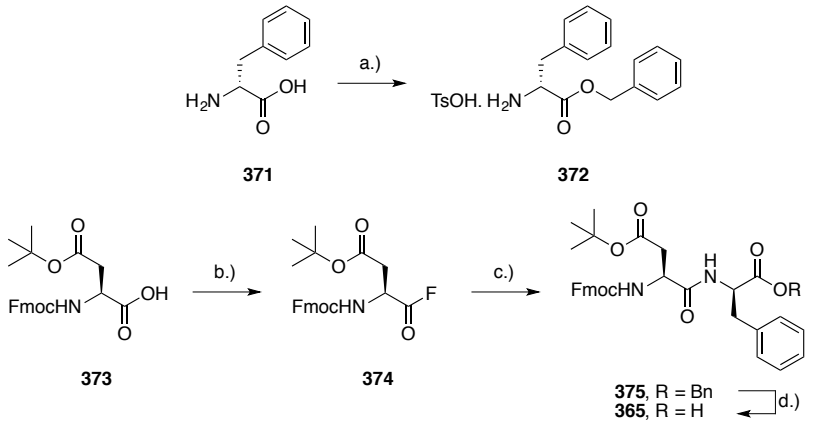
Fragment A **364** was prepared in five steps from glycine **366** as illustrated in Scheme 102.



Scheme 102: *Reagents and conditions*: a.) Allyl alcohol, *p*-TsOH, toluene, reflux (Dean-Stark), 16 h, 90%; b.) Fmoc-Arg(Pbf)-OH, HOBt, EDCI.HCl, DMF, 0 °C to r.t., 18 h, 89%; c.) piperidine, DMF, r.t. 0.25 h, 95%; d.) **321**, HATU, CH_2Cl_2, r.t. 18 h, 87%; e.) piperidine, DMF, r.t. 0.5 h, 82%.

Glycine **366** was protected as its allyl ester **377** in a condensation reaction using allyl alcohol, and *p*-TsOH in toluene under Dean-Stark conditions (Scheme 102).[378] Purification by recrystallisation from dichloromethane furnished allyl glycine **377** as the tosylate salt in 90% yield. Amide coupling with Fmoc-Arg(Pbf)-OH using EDCI.HCl and HOBt in DMF allowed the preparation of dipeptide **368** in 89% yield with an optical rotation of -5.3° (*c* = 1, $CHCl_3$) (Scheme 102). Deprotection of the Fmoc group of **368** using piperidine in DMF furnished amine **369** in 95% yield. Further coupling with Fmoc-amino acid **321** using HATU provided tripeptide **370** in 87% yield. This was efficiently deprotected to give the free amine **364** in 82% yield (Scheme 102).

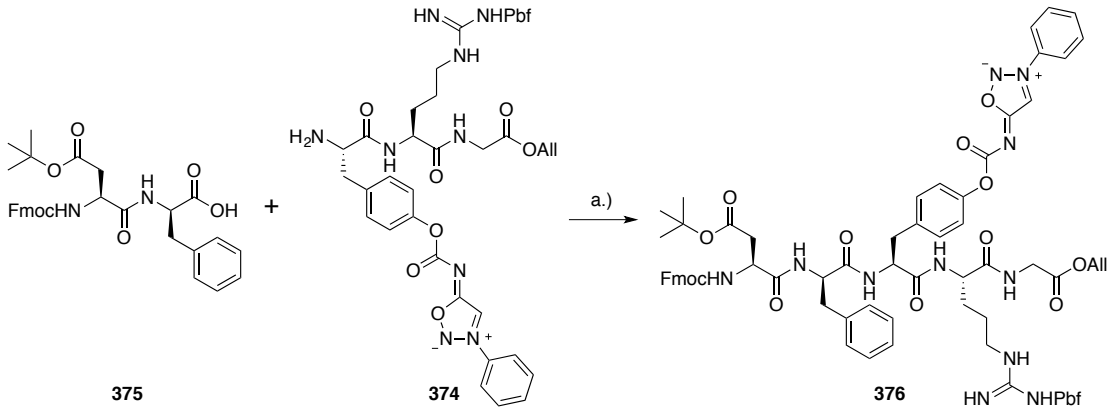
Fragment B **365**, was prepared following the literature procedure of Carpino *et al* (Scheme 103).[379,380]



Scheme 103: *Reagents and conditions*: a.) Allyl alcohol, *p*-TsOH, toluene, reflux (Dean-Stark), 16 h, 81%; b.) Cyanuric fluoride, pyridine, 0 °C to r.t., 4 h, 94%; c.) **372**, $(iPr)_2EtN$, CH_2Cl_2,, 0 °C to r.t., 16 h, 68%; d.) 10% Pd/C, ammonium formate, r.t., 3 h, 85%.

Thus, D-phenylalanine **371** was converted to its benzyl ester **372** (Scheme 103).[378] Activation of Fmoc-Asp(OtBu)-OH **373** to the corresponding acyl fluoride using cyanuric fluoride provided **374** in 94% (Scheme 103). The formation of the acyl fluoride was confirmed by ^{19}F NMR with a signal at +28.2 ppm. A condensation could then be achieved between amino acids **372** and **374** to furnish dipeptide **375** (68% yield) (Scheme 103). Dipeptide **375** could also be prepared using HOBt and EDCI in DMF. The yield for both methods is similar. The sequence was completed with a transfer hydrogenation using ammonium formate and Pd/C which furnished carboxylic acid **365** in 85% yield after chromatography (Scheme 103).

With the two fragments in hand, dipeptide **375** and tripeptide **374** were coupled together using HATU to provide the peptapeptide **376.** This coupling was achieved in 82% yield and as single diastereoisomer after chromatography (Scheme 104).

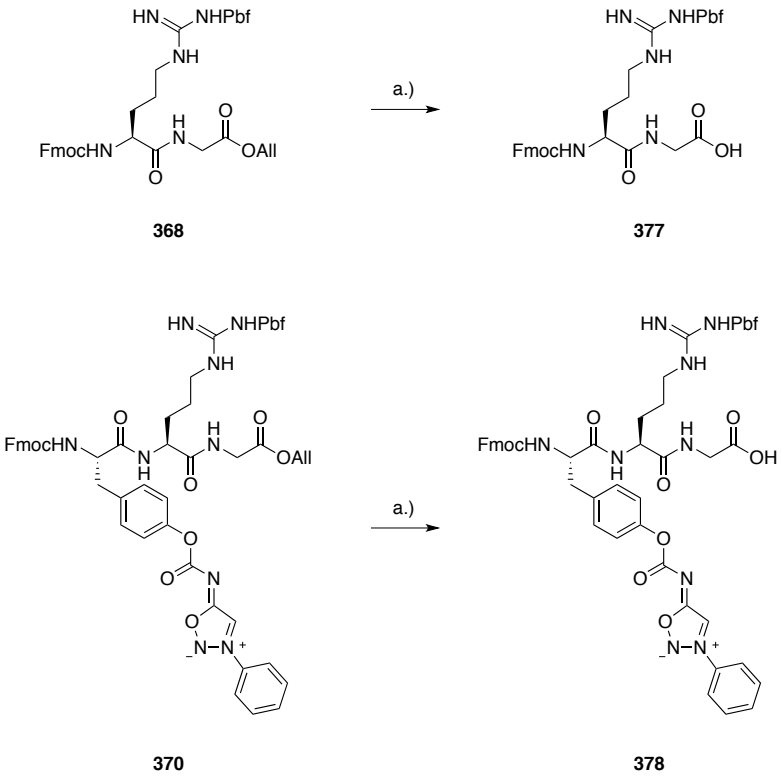


375 374 376

Scheme 104: *Reagents and conditions*: a.) HATU, CH_2Cl_2, $(iPr)_2EtN$, 0 °C to r.t., 18 h, 82%.

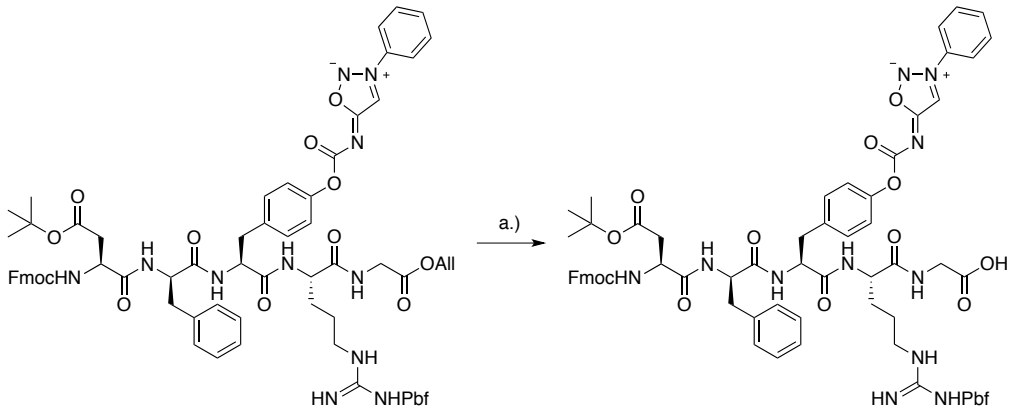
With the protected linear pentapeptide **376** in hand, deprotection of the allyl ester was considered. The deprotection was explored using $Pd(PPh_3)_4$ and morpholine as an allyl scavenger based on conditions previously reported for deprotection of similar glycyl allyl esters in peptides.[381,382] This did not however yield the desired product. Consumption of the starting ester was observed, but the carboxylic acid could not be isolated. The use of morpholine as the allyl scavenger was identified as the problem in this reaction, as morpholine can also be used to deprotect Fmoc groups. As such dimedone was explored as an alternative allyl scavenger.

Two model substrates were used to explore the using dimedone/$Pd(PPh_3)_4$ deprotection these were the dipeptide **368** and tripeptide **370** (Scheme 105).



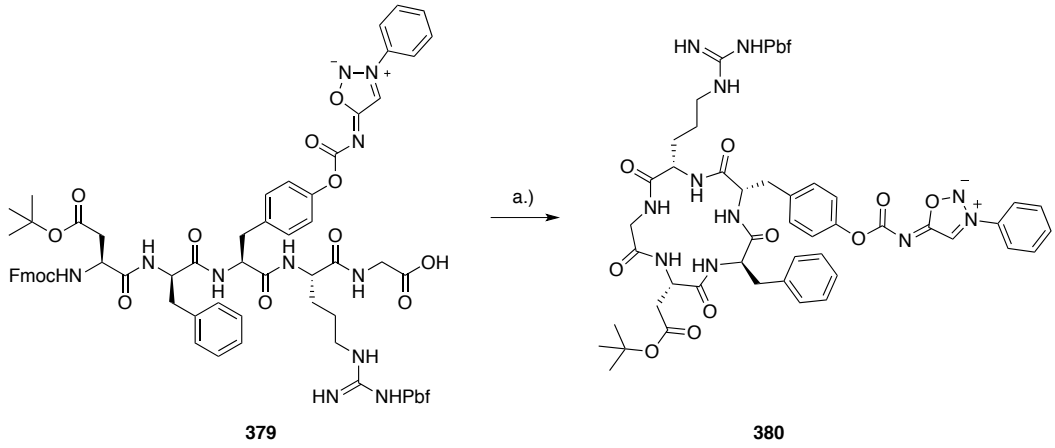
Scheme 105: *Reagents and conditions*: Dimedone, $Pd(PPh_3)_4$, dry THF, r.t., 3-5 h, **377** = 95%, **378** = 88%.

These two peptides appeared appropriate as they possess the same sequence of amino acids as found in pentapeptide **376**. Fortuitously when they were treated with dimedone (3-5 molar equiv.) and $Pd(PPh_3)_4$ (5-8 mol%) in dry THF, the expected carboxylic acids were isolated in 95% and 88% yield respectively, with no observed racemisation. These conditions were applied to pentapeptide **376** and this also proved successful furnishing the pentapeptide acid **379** in a 77% yield (Scheme 106).



Scheme 106: *Reagents and conditions*: Dimedone, $Pd(PPh_3)_4$, dry THF, r.t., 3-5 h, 77%.

With carboxylic acid **379** in hand, attention turned to deprotection and then macrolactamisation. The literature reports many examples of peptide macrolactamisation in natural product total synthesis.[383–385] It was chosen to mediate the Fmoc deprotection using piperidine in DMF, and then use the unpurified material directly in the macrolactamisation reaction (Scheme 107, Table 23, Entry 1).

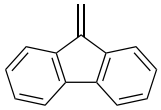


Scheme 107: *Reagents and conditions*: see **Table 23**.

Entry	Fmoc deprotection conditions	Macrolactamisation conditions	Outcome
1	Piperidine/DMF, 0.5 h	HATU, CH_2Cl_2, 0.01 M, 36 h	9-methylenefluorene isolated
2	Et_2NH/CH_3CN, 3 h	N/A	no product isolated
3	Piperdine/CH_2Cl_2, 0.5 h	HATU, HOAt, DMF, 0.01 M, 24 h	no product isolated
4	Piperidine/CH_2Cl_2, 0.5 h	HATU, HOAt, CH_2Cl_2, 0.001 M, 36 h	9-methylenefluorene isolated
5	Piperidine/CH_2Cl_2, 0.5 h	T3P®, $(iPr)_2NEt$, CH_2Cl_2, 0.001 36 h	9-methylenefluorene isolated
6	PS-Piperazine, CH_2Cl_2, 24 h	T3P®, $(iPr)_2NEt$, CH_2Cl_2, 0.001 M, 72 h	no product isolated

Table 23: Conditions explored for the macrolactamisation of **379**.

Deprotection of the Fmoc group was monitored by thin-layer chromatography, but the corresponding product could not be observed on TLC. ^{1}H NMR indicated some "peptide-like" signals, and as such the crude reaction product was subjected to macrolactamisation conditions with HATU, but only 9-methylenefluorene **381** could be isolated.



381

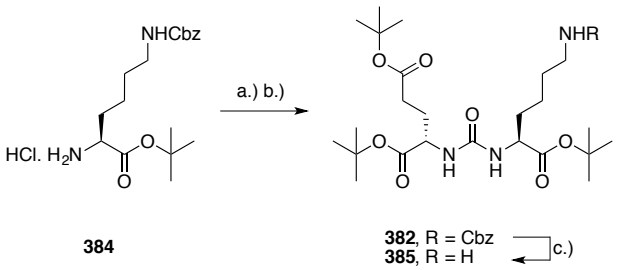
Based on this result a series of attempts were made to isolated the intermediate amino acid and purify it by column chromatography (Table 23, Entry 2). However in all cases the product could not be isolated. The presence of the amino acid could not be determined by thin-layer chromatography, UV or ninhydrin visualisation on normal or C18 reverse-phase silica plates. As a result the deprotection and macrolactamisation

were undertaken without any purification of the intermediate amino acid, beyond solvent evaporation and drying under vacuum. A solution of piperidine in dichloromethane was identified as the optimum conditions for Fmoc deprotection (Table 23, Entry 3-5), as the residual solvent was readily removed. Three macrolactamisation conditions were trialled using HATU and T3P®; but the product was not observed in either case. Polymer-supported piperazine was also explored used in place of piperidine (Table 23, Entry 6). Deprotection of the Fmoc group was apparent, however when subjected to macrolactamisation the lactam was not formed. Macrolactamisation of peptide **389** is clearly proving difficult and could not be achieved during the course of this research.

5.4 NITRIC OXIDE DONATING PSMA INHIBITORS-RESULTS AND DISCUSSION

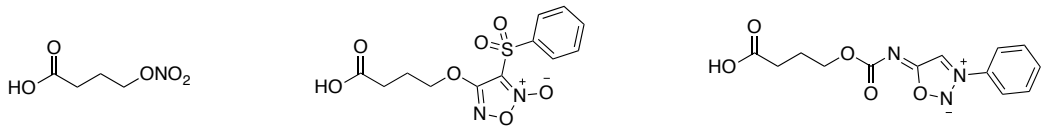
Chapter Two described the use of a simple heterodimeric urea scaffold to target and inhibit PSMA for radioimaging.[256,257] This urea was identified as a potential homing unit for drug delivery and NO location. We sought to prepare similar urea-based PSMA inhibitors appended with our NO release units previously described *i.e*. nitrate esters, furoxans and sydnonimines. In addition, we aimed to combine a PSMA inhibitor with the CYP17 inhibitor, abiraterone, and an NO release sydnonimine.

The Glu-Lys urea **382** was prepared from commercially available di-*tert* butyl protected glutamic acid **383**, and ε-Cbz *tert*-butyl lysine **384** in a protocol similar to that reported by Maresca *et al.* (Scheme 108).[257] Generation of the isocyanate of **384** followed by its reaction with **383** generated protected urea **382**. Hydrogenolysis of the Cbz group was carried out in a flow reactor (H-Cube) in order to furnish the amine **385** in quantitative yield (Scheme 108).

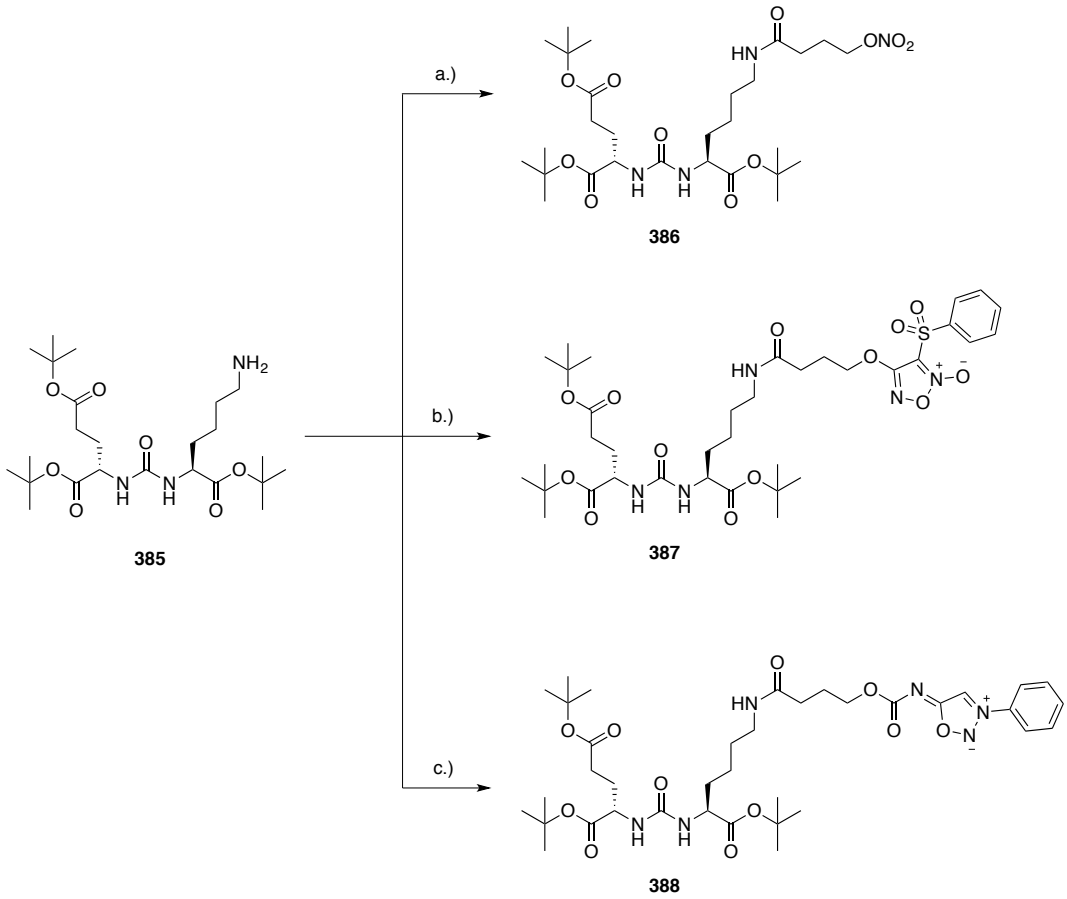


Scheme 108: *Reagents and conditions*: a.) Triphosgene, $(iPr)_2EtN$, dry CH_2Cl_2, -20 °C, 1 h; b.) **383**, $(iPr)_2EtN$, 2 h, -20 °C to r.t., 78%; c.) H-Cube, 1 atm pressure, 1 mL/min, r.t., 10% Pd/C cartridge, quant..

Maresca *et al*. have reported the use of amine **385** in reductive aminations, isocyanate condensation and sulfonamide preparation.[257] We chose this amine to link to carboxylic acids **258**, **261** and **266** as previously described in Chapter Three.

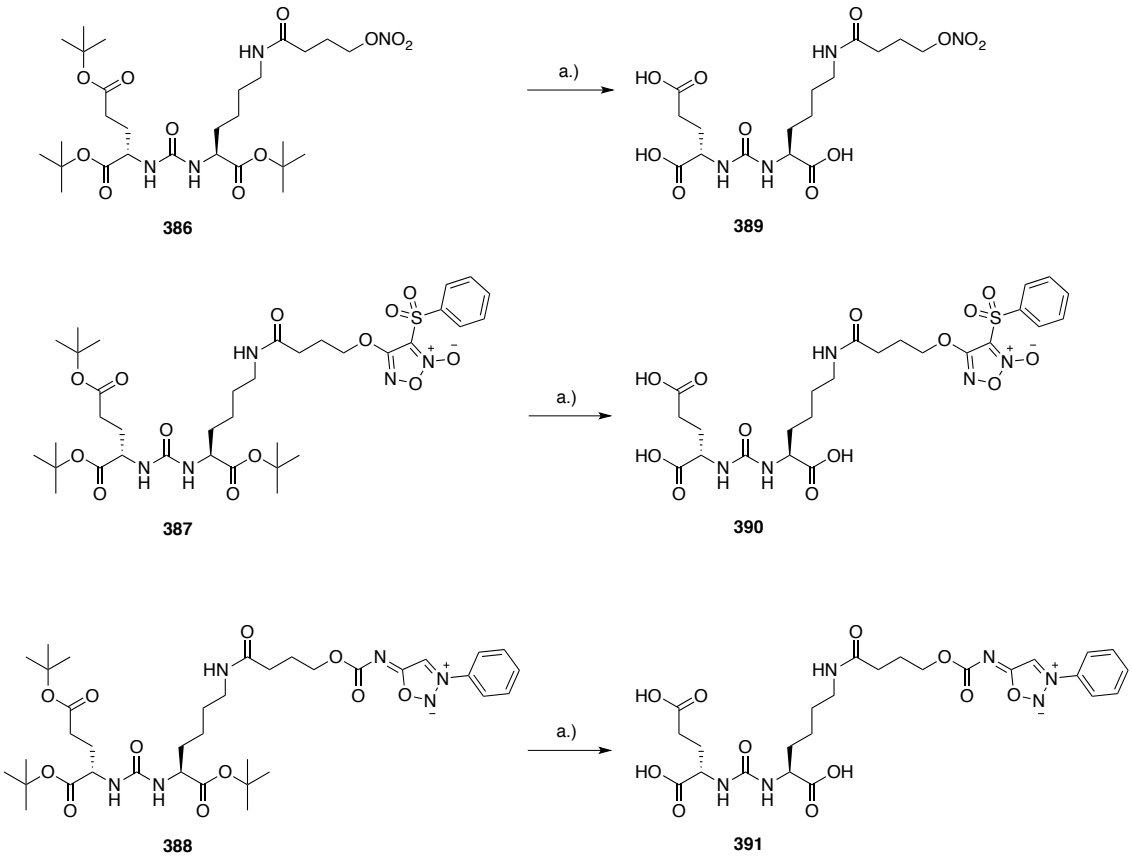


To this effect, amide coupling between acids **258**, **261** and **266** with amine **385** using HATU as a coupling reagent furnished the desired amides **386-388** in excellent yield (Scheme 309).



Scheme 309: *Reagents and conditions*: a.) **258**, HATU, $(iPr)_2EtN$, DMF, r.t. 18 h, 70%; b.) **261**, HATU, $(iPr)_2EtN$, DMF, r.t. 18 h, 72%; c.) **266**, HATU, $(iPr)_2EtN$, DMF, r.t. 18 h, 66%.

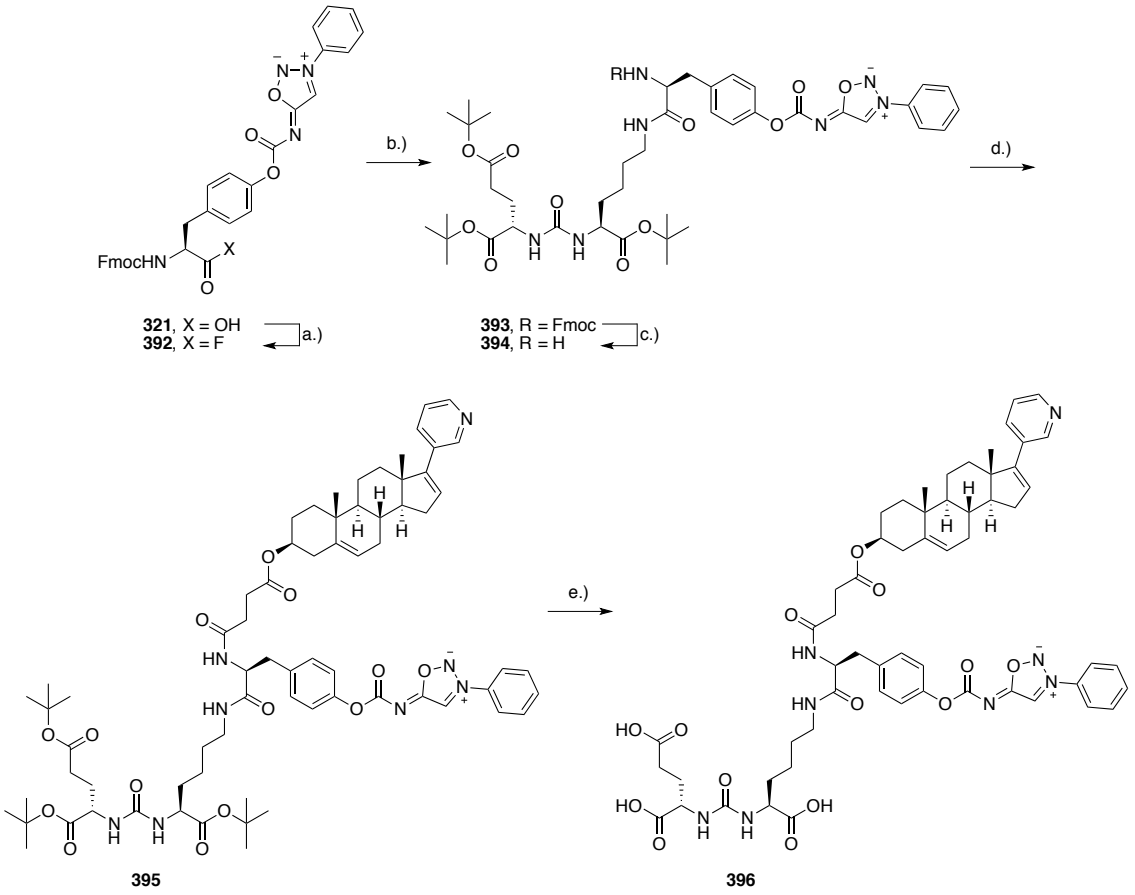
Deprotection of the *tert*-butyl esters **386-388** using TFA and purification by trituration with acetonitrile provided the carboxylic acids **389-391** (Scheme 310). These PSMA inhibitors were insoluble in all solvents, apart from TFA and DMSO and as a result it was not possible to obtain optical rotations on these molecules. The poor solubility also has implications for their potential use as NO donors in a clinical setting. Their may be improved by replacing the amide with a bioisostere with greater solubilising potential, such as a sulfonamide or secondary amine. The solubility may also be improved by adding appropriate groups to the appended carboxylic acids. These avenues remain to be explored.



Scheme 310: *Reagents and conditions*: a.) TFA, CH_2Cl_2, 0 °C, to r.t., 18 h, **389** = 90%, **390** = 92%, **391** = 80%.

In addition to the NO-conjugates **389-391**, the PSMA inhibitor motif was also developed as a component in an NO and abiraterone **106** conjugate.

Conversion of amino acid **321**, as previously prepared in Chapter Five, to the corresponding acyl fluoride **392**, and subsequent condensation with amine **385**, provided the Fmoc-protected peptide **393**. Fmoc deprotection with piperidine furnished amine **394**, which was then linked with abiraterone hemisuccinate **352** to furnish amide **395**. Finally, TFA deprotection followed by purification by trituration with acetonitrile gave the *tris*-acid **396** (Scheme 311).



Scheme 311: *Reagents and conditions*: a.) Cyanuric fluoride, pyridine, CH_2Cl_2, 0 °C to r.t., 4 h, quant., b.) **385**, $(iPr)_2EtN$, CH_2Cl_2,, 0 °C to r.t., 16 h, 78%; c.) Piperdine, DMF, 0 °C to r.t., 0.5 h, 90%; d.) **352**, HATU, $(iPr)_2EtN$, DMF, r.t. 18 h, 91%; e.) TFA, CH_2Cl_2, 0 °C to r.t., 18 h, 88%.

5.4.1 NITRIC OXIDE RELEASE

The Griess test was used to determine the level of NO release from the NO-PSMA inhibitors and the results are summarised in Table 24.[188] PSMA inhibitors **389-391** and **396** (1 mmol, [final], 400 μM) in of DMSO were incubated with a GT buffer containing SOD ([GT] 6 mmol, final [GT] 600 μM, pH 7.6, [SOD] 4557 U mL^{-1}).

Compound	NO_2^- production (µmol) (GST buffer)	NO_2^- production as % (GST buffer)	NO_2^- production (µmol) (pH 7.6 buffer)	NO_2^- production as % (pH 7.6 buffer)
389	47.7 ± 2.2	11.4	2.9 ± 1.6	<1.0
390	57.2 ± 4.4	14.3	3.3 ± 1.0	<1.0
391	22.0 ± 2.9	5.5	1.9 ± 0.4	<1.0
396	33.9 ± 6.1	8.5	1.8 ± 0.5	<1.0

Table 24: NO release from PSMA inhibitors. Data are the averages of triplicate experiments

PSMA inhibitors **389-391** and **396** all displayed NO release properties in the presence of GT. The highest level of NO released was observed from furoxan **390** (57.2 ± 4.4 µM, 14.3 %) released. The NO release from the abiraterone conjugate **396** was also significant (33.9 ± 6.1 µM, 8.5%). NO release in pH 7.6 buffer was less than 1% in each case.

The targeting of NO delivery with PSMA inhibitors presents as an attractive option for prostate cancer therapy and these initial studies pave the way for future work on compound optimisation.

CHAPTER SIX: CONCLUSIONS AND FUTURE WORK

Through the course of this thesis NO-donating functionalities have been applied in the synthesis of an array of bioactive molecules, varying from conjugation with drug molecules to installation in amino acids and peptides.

A total of 47 analogues of NCX-1102 were prepared, using nitrate esters, furoxans and sydnonimines as NO-donating functional groups. These were screened for cytotoxic activity at the University of Edinburgh, and two lead compounds, **181** and **183** were identified for further test in a mouse model of prostate cancer, the work on the sulindac series of compounds has been submitted for publication in *Bioorganic and Medicinal Chemistry*. This strategy was then applied to abiraterone, a CYP17 inhibitor recently licenced for the treatment of CRPCa; in this study, a furoxan analogue **253** was selected as a lead compound. Further biological evaluation is underway on this series of compounds at the University of Edinburgh.

In addition to NO-drug hybrids, the concept was extended to amino acid motifs. With a series of tyrosine and serine derived NO-donating amino acids prepared. This work was published in *Organic and Biomolecular Chemistry* in 2013.[386] These amino acids were employed in the synthesis of NO-donating RGD peptides, and PSMA inhibitors. The linear RGD peptides displayed NO release in the presence of glutathione, and had similar affinity towards the $\alpha_v\beta_3$ integrin. Further biological assays into the potential cytotoxicity of these peptides are being undertaken at the University of Aberdeen.

Overall this research has examined the delivery of NO using drug hybrids and through more targeted means, such as amino acids and peptides. The delivery of NO to a target organ is a diffult task. The appending of NO release units to known molecules, with known activities is at first glance, an easy way of delivering NO along with the drug

molecule. But this is not guaranteed, NO release is difficult to control and this is the main challenge for developing targeted therapies.

The final part of this thesis discusses the possible future work that could be undertaken to continue this research.

Three possible aspects of work have been described herein, these are:

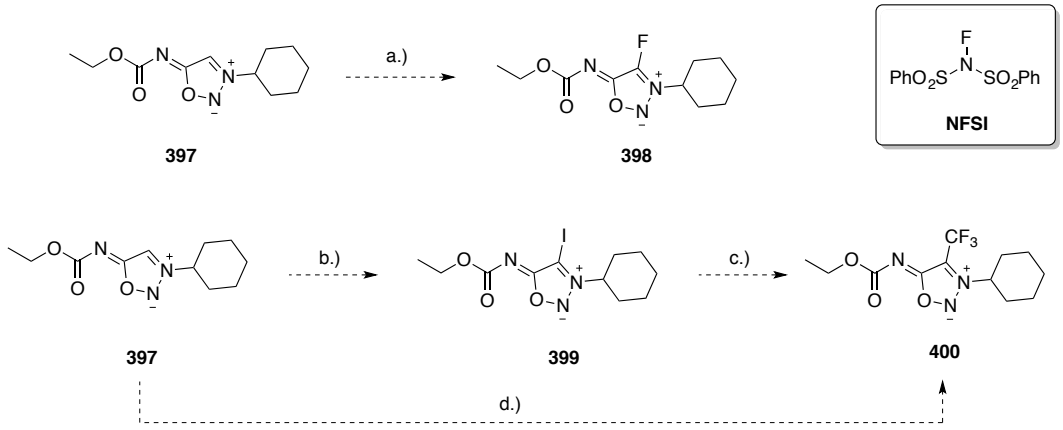
1.) Fluorinated sydnonimines

2.) Imaging PSMA expression with positron emission tomography.

3.) Simple highly-functionalised NO-donating linkers for bioconjugation.

6.1 FLUORINATED SYDNONIMINES

As shown in Chapter 1, halogenation of the C4 position of the sydnonimine ring is easily accomplished for chlorine, bromine and iodine using the corresponding *N*-halogen succinimide (Chapter 1, Scheme 21, page 36).[169] Installation of a fluorine atom in C4 is not known. Given the prevalence of fluorinated molecules in organic chemistry, particularly in relation to pharmaceuticals, a fluorinated syndonimine would be a valuable functional group. To this end, the synthesis of two fluorinated sydnonimines is proposed (Scheme 312).



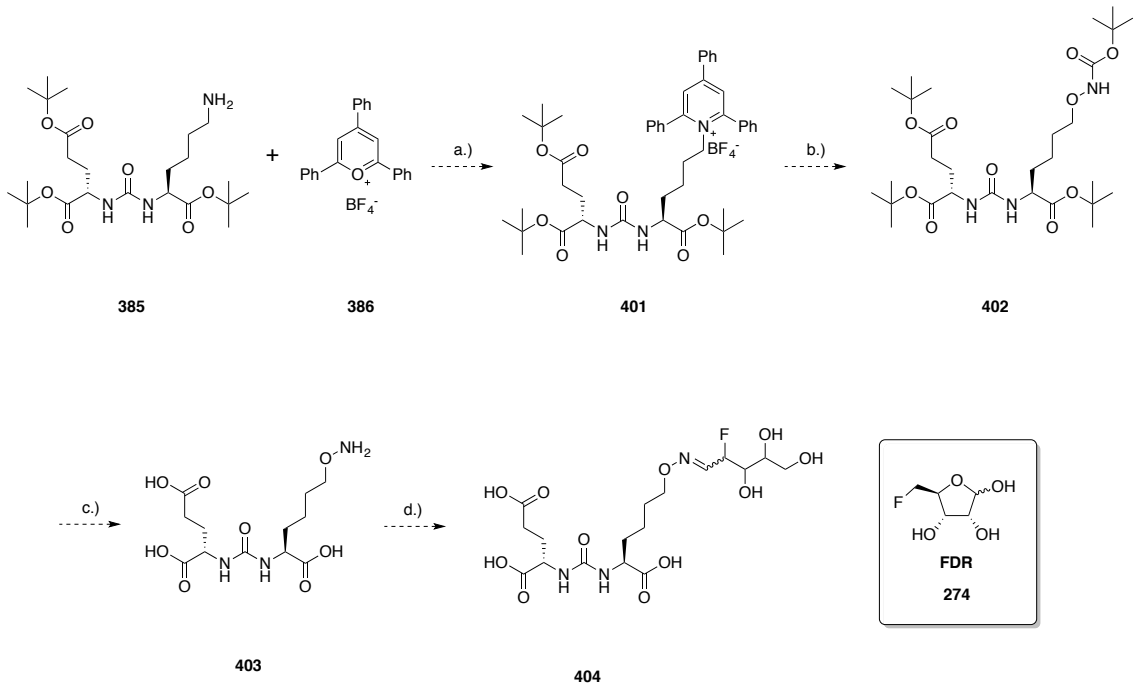
Scheme 312: Fluorination of sydnonimines. *Proposed reagents and conditions*: a.) i.) *n*-BuLi,; ii.) NFSI; b.) NIS; c.) CF_3SO_2Cl, $Ru(phen)_3$, *hv*; d.) $NaSO_2CF_3$ (Langois reagent), tBuOOH, CH_2Cl_2:H_2O.

Sydnonimine **397** has been previously prepared in our group. In this proposed synthesis, the fluorination of **397** is proposed by lithiation and fluorination of the anion with an electrophilic fluorination reagent such as NFSI to provide fluorosydnonimine **398**. Trifluoromethylation of **397** is forseen to occur in a two-step process; initial iodination with NIS to sydnonimine **399**, followed by trifluoromethylation by photoredox catalysis to give trifluoromethylsydnonimine **400**, based on the work of MacMillan.[387] This method of trifluoromethylation has been shown to work with a wide range of aromatic and heteroaromatic systems; and could be easily applied using commercially available reagents. Direct trifluromethylation of **397** may also be possible using the trifluoromethylation conditions developed by Baran using the Langois reagent ($NaSO_2CF_3$).[388]

With these in hand, it would be interesting to examine the kinetics of hydrolysis to release NO with the fluorinated sydnonimines **298** and **400**, and parent sydnonimine **397**. This could be undertaken using ^1H and ^{19}F NMR spectroscopy.

6.2 IMAGING PSMA EXPRESSION WITH POSITRON EMISSION TOMOGRAPHY

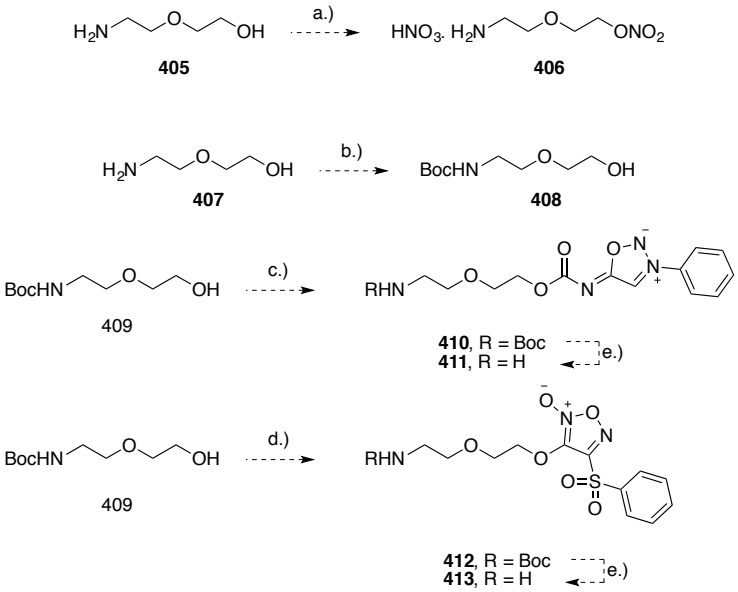
Positron emission tomography (PET) is a developing technique for medical imaging. As described in Chapter Five, research within the group has focused on the use of fluorine as a radiotracer for PET imaging, using the RGD sequence to image integrin expression with fluorodeoxyribose as the source of fluorine.[367,389,390] The PSMA inhibitor motif described in Chapter Five has been used as a method of imaging PSMA expression in prostate cancer tumour tissue. This has involved using radiolabelled iodine,[257] technetium[391] and rhenium.[392] Based on work in the group, a four-step synthesis is proposed to develop a labeled PSMA inhibitor using FDR as the fluorine source (Scheme 313). Conversion of the amine **385**, prepared previously, to the triphenylpyrillium salt **401** gives a leaving group suitable to be substituted with *N*-Boc hydroxylamine to give *tert*-butoxycarbonyl protected material **402** (Scheme 313). Global deprotection of the *tert*-butoxycarbonyl and *tert*-butyl esters with TFA would provide **403**. This would be a suitable precursor for oxime bioconjugation with FDR to give labelled inhibitor **404** (Scheme 313).



Scheme 313: Synthesis of fluorinated PSMA inhibitor. *Proposed reagents and conditions*: a.) Ethanol, Δ; b.) *N*-Boc hydroxylamine; c.) TFA; d.) **274** (FDR), Buffer.

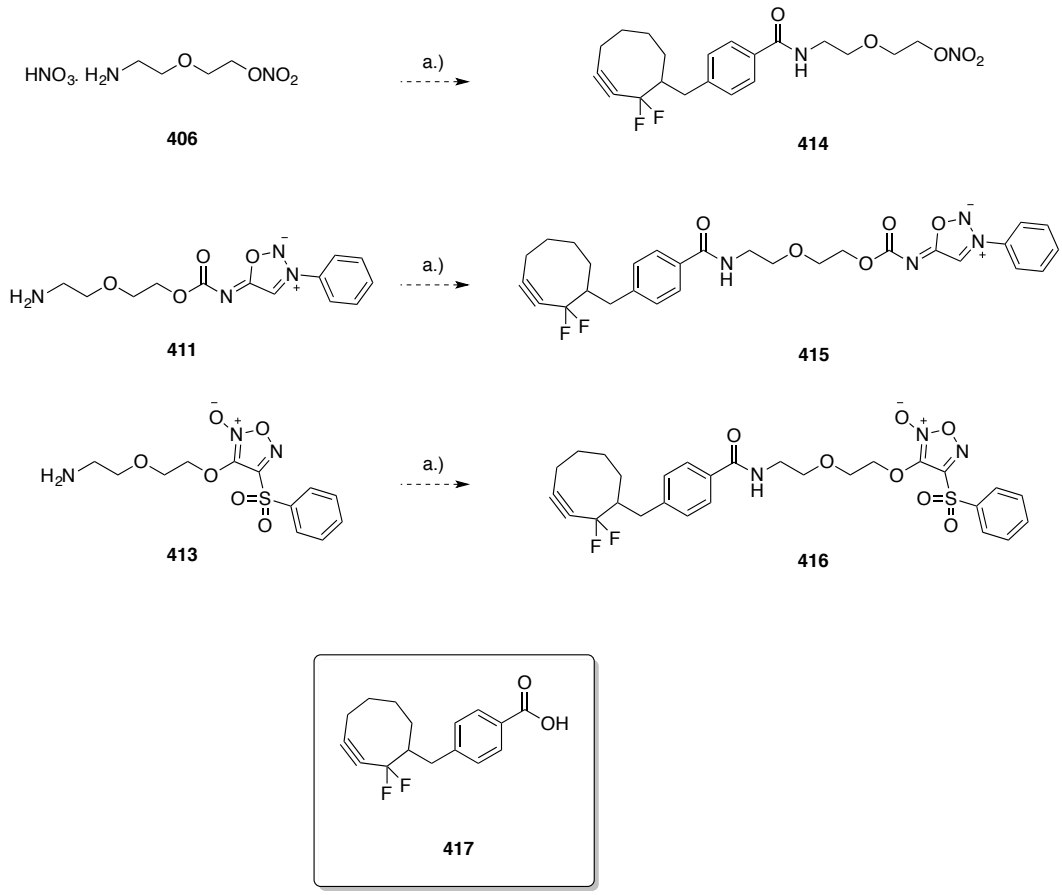
6.3 SIMPLE HIGHLY-FUNCTIONALISED NO-DONATING LINKERS FOR BIOCONJUGATION.

Bioconjugation reactions represent a simple method of the post-translational modification of biomolecules. To this effect, the development of a "tool-box" of bifunctionalised linkers is proposed. These would contain an NO-release unit and a reactive site for bioconjugation. These would all be accessed from a series of amines, with nitrate ester, furoxan and sydnonimine functionalities (Scheme 314).



Scheme 314: *Proposed reagents and conditions*: a.) HNO_3, Ac_2O; b.) Boc_2O, Et_3N, $CHCl_3$; c.) **218**, CH_3CN, Δ; d.) **53**, DBU; e.) TFA, CH_2Cl_2.

Nitrate ester **406** could be prepared based on a literature procedure[393] from 2-(aminoethoxy)ethanol **405** (Scheme 314). Boc-protected amine **408**[394] could be used to access sydnonimine **411** and furoxan **413**. Amines **406**, **411** and **413** could be functionalised to the corresponding azide using TfN_3**.** The corresponding alkynes **414-416** could be prepared *via* an amide coupling between amine **406**, **411** or **413** and the second generation di-fluoro cyclooctyne **417** developed by Bertozzi *et al*.[395] These azides and alkynes could be used in azide-alkyne Huisgen cycloadditions.



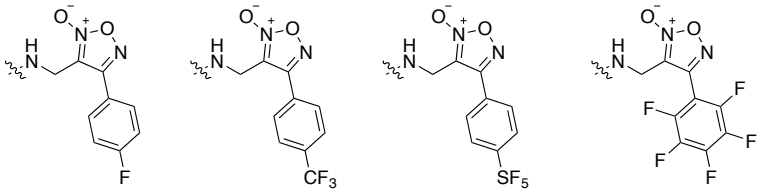
Scheme 315: *Proposed reagents and conditions*: a.) EDCI.HCl, CH_2Cl_2.

In a similar fashion, the *N*-hydroxysuccinyl carbamates could be prepared for lysine ligation.

In combination, this "tool-box" would allow for a wide variety of bioconjugation reactions to take place on a range of chemical functional groups. While it would be possible to prepare similar linkers with a maleimide functional group for thiol-ene type ligation, this would be counterproductive for bioconjugation with these groups, as the sulfhydryl groups are required for the reaction with the NO-donating functional group to release NO.

However, this reactivity could be used to develop a bioconjugation reaction that released NO upon bioconjugation. For example, thiophilic bioactivation of furoxans generates a covalent C-S bond in the NO release mechanism.[139] It has been

demonstrated that electron-withdrawing groups promote the reaction with thiolate ions,[157] and as such, a furoxan with an increased number of electron-withdrawing groups, such as fluorine groups *e.g* **418-421**, could be an alternative to maleimides for bioconjugation chemistry, combined with release of nitric oxide.



418 **419** **420** **421**

APPENDIX: EXPERIMENTAL SECTION

A.1 GENERAL EXPERIMENTAL PROCEDURES

For anhydrous reactions, glassware was flame dried under high vacuum. Reactions were carried out under an atmosphere of argon, unless otherwise noted. Compressed argon was passed through a drying column packed with 4 Å molecular sieves, potassium hydroxide and self-indicating desiccant, before reaching a double manifold. Dry CH_2Cl_2 and THF were obtained by sequential passage through drying columns, followed by dispensing under an atmosphere of argon from an mBRAUN SPS-800 solvent purification machine, THF was used unstabilised. Dry acetonitrile was obtained by drying over CaH_2 followed by distillation under argon and stored over CaH_2. Dry solvents were used when indicated in the procedure.
All chemicals were purchased from: Sigma Aldrich, Alfa Aesar, Fluorochem, TCI Europe or Fisher Scientific. Silica Gel 60 (240–400 mesh) was purchased from Merck. Glass-backed TLC plates with 254 nm indicator were also purchased from Merck. Flash column chromatography was carried out following the procedure reported by Still *et al*.[396]
Triethylamine, diisopropylamine and piperidine were distilled from KOH before use and stored over KOH. Any chemicals requiring further purification were purified by the literature method.[397]

^{1}H NMR spectra were recorded 300, 400 or 500 MHz Bruker Avance/Avance II/Avance III spectrometers. All spectra were acquired in deuterated solvents, and calibrated to the residual chemical shift of that solvent. Chemical shifts are given in δ as in units of parts per million (ppm) relative to tetramethylsilane. Proton assignments are made according to chemical shift, multiplicity and 2D NMR experiments and are reported to 2 decimal place. Coupling constants (*J*) are reported to nearest 0.1 Hz. NMR spectra were interpreted using TopSpin v3.0.

^{13}C NMR spectra were recorded at 75, 101, 126 MHz on Bruker Avance/Avance II/Avance III spectrometers. Resonances were assigned by reference to DEPTQ, HMBC and HSQC spectra and are reported to 1 decimal place with coupling constants reported to nearest 0.1 Hz.

^{19}F NMR spectra were recorded at 376 MHz on Bruker Avance II spectrometer. Fluorine assignments are made according to chemical shift, multiplicity, and reference to the literature. Coupling constants are reported to 0.1 Hz and are averaged for coupling nuclei.

Multiplicities are as follows: s – singlet, br s – broad singlet, m – multiplet, d – doublet, dd – doublet of doublets, ddd – doublet of doublet of doublets, dt – doublet of triplets, t – triplet, q – quartet.

Under reduced pressure refers to the use of a KNF diaphragm pump or Büchi V-700 vacuum pump to remove solvent under reduced pressure on a Büchi Rotavapour R-210 apparatus, with a water bath at 40 ºC and a Büchi V-850 pressure regulator. The bath temperature was reduced to 0 ºC with ice when removing solvents from volatile compounds. Drying under vacuum refers to the use of an Edwards RV-3 rotary vane oil pump at a pressure of <0.1 mbar.

Lyophilisation refers to the removal of water by sublimation on a Christ Alpha 1-2 LD plus freeze dryer equipped with an Edwards RV-3 two stage rotary vane oil pump.

Optical rotations were recorded on a Perkin Elmer model 341 polarimeter with a cell pathlength of 1 dm. Samples were recorded at 589 nm (sodium D-line). Concentrations are reported in g/100 mL and specific optical rotations are denoted as $[\alpha]_{\lambda}^{20}$ in the implied units of 10^{-1} deg cm^3 g^{-1}.

TLC plates were visualised under UV light (254 or 365 nm) and/ or stained with the appropriate staining solution The staining solution is reported when used: either basic aqueous potassium permanganate or ethanolic cerium phosphomolybdate.

Melting points were measured using a Gallenkap Griffin MPA350 melting point apparatus and are uncorrected.

Mass Spectroscopic analyses at the School of Chemistry were conducted by Mrs Caroline Horsburgh on a Micromass LCT electrospray time of flight mass spectrometer. Samples sent to the EPSRC mass spectrometry service in Swansea were analysed on a Thermofisher LTQ Orbitrap XL mass spectrometer using electrospray ionisation (ES). *m*/*z* values are reported in Daltons.

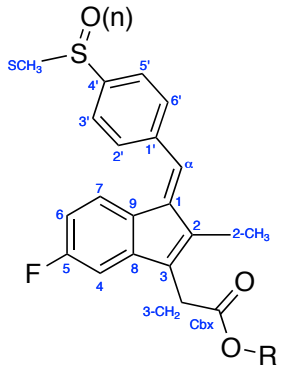
IR spectra were recorded a Perkin Elmer Spectrum GX FT-IR machine as either a KBr disk, or as thin films on NaCl plates.

Elemental microanalyses were performed on a Carlo-Erba EA 1108 with PC based data system, Eager 200 for Windows and a Sartorious Ultra Micro Balance, 4504MP8 at the UCL London School of Pharmacy.

Miscellaneous Brine refers to a saturated solution of sodium chloride in distilled water.

A.2 COMPOUND NUMBERING

Compounds based on the sulindac **112** core structure are numbered in the experimental section based on the style reported by Douglas.[398] Primes are used when necessary.



Compounds based on the abiraterone **106** core structure are numbered in the experimental section based IUPAC nomenclature for steroids. Primes are used when necessary.

Amino acids and peptides are numbered on the structures sequentially, not based on IUPAC nomenclature, for ease. Primes are used when necessary.

A.3 GENERAL PROCEDURES

General procedure A

EDCI.HCl (1-2 equiv.), the alcohol (1 equiv.) and DMAP (10 mol%) were added to a solution of sulindac derivative (sulfoxide, sulfide or sulfone, 1 equiv.) in dichloromethane (10 mL) and stirred for 4 h at room temperature. The solution was partitioned with aq. HCl solution (2 N, 10 mL). The organic layer was separated and washed with aq. HCl solution (2 N, 5 mL) and brine (10 mL). The organic layer was dried over $MgSO_4$ and the solvent removed under reduced pressure to yield the crude material. This crude material was purified by silica gel chromatography to give the desired ester.

General Procedure B

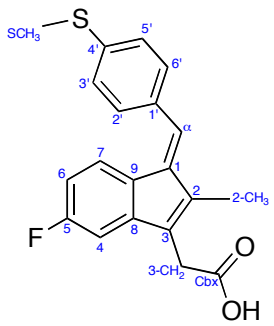
HATU (3 equiv.) was added to a solution of amine (1 equiv.) and acid (1.5 equiv.) in dry DMF (5 mL) cooled to 0 °C. The solution was stirred for 15 min and diisopropylethylamine (distilled, 4 equiv.) was added. The solution was allowed to warm to room temperature and stirred for 18 h. The resultant solution was diluted with ethyl acetate (50 mL) and water (50 mL). The organic layer was separated and washed with aq. HCl (2 N, 20 mL), aq. Na_2CO_3 (saturated, 20 mL) and brine (20 mL). The organic layer was separated and dried over Na_2SO_4, filtered and the solvent removed under reduced pressure. The resultant residue was uptaken in dichloromethane and adsorbed onto silica gel. Purification by silica gel chromatography provided the desired amide.

General procedure C

RGD peptide (upto 100 mg) was dissolved in a deprotecting cocktail of TFA:TIPS:H_2O (700 μL, 95:2.5:2.5) and stirred for 2 h. The solvent was removed under reduced pressure and the residue triturated with cold diethyl ether. The residue was dissolved in *tert*-butanol:H_2O (5 mL, 1:4), snap-frozen and lyophilised to give the trifluoroacetate salt.

A.4 EXPERIMENTAL PROCEDURES FOR CHAPTER THREE

(*Z*)-2-(5-Fluoro-2-methyl-1-(4-(methylthio)benzylidene)-1*H*-inden-3-yl)acetic acid (sulindac sulfide), 115[308]

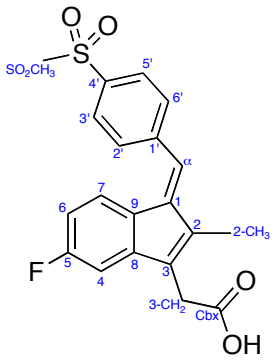


Titanium (IV) chloride (1.0 M in CH_2Cl_2 solution, 1.5 mmol, 1.5 mL) and PS-triphenylphosphine (3.00 mmol/g loading, 1.5 g, 1.5 mmol) were added to a solution of sulindac **112** (356 mg, 1.00 mmol) in dry tetrahydrofuran (20 mL) at 0 °C. The mixture was allowed to warm to room temperature and stirred for 24 h. After this time, the suspension was filtered through Celite and the resulting solution quenched with aq. $NaHCO_3$ solution (saturated, 100 mL). The aqueous layer was extracted with diethyl ether (3 × 30 mL). The combined ether layers were washed with brine (50 mL), dried over Na_2SO_4, filtered and the solvent removed under reduced pressure to yield (*Z*)-2-(5-fluoro-2-methyl-1-(4-(methylthio)benzylidene)-1*H*-inden-3-yl)acetic acid, sulindac sulfide **115,** (320 mg, 94%, 0.94 mmol) as a yellow solid, which was used without any further purification: **m.p.** 186-188 °C, [Lit.[308] 186-190 °C]; $\boldsymbol{\nu_{max}}$ (thin film)/cm^{-1} 2926, 1702, 1557, 1601, 1491, 1048; **^{1}H NMR** (500 MHz; $CDCl_3$) δ 7.44 (2H, d, *J* 8.2 Hz, C*H*-2′,6′), 7.36 (1H, dd, *J* 8.4, 5.3 Hz, C*H*-7), 7.29 (2H, d, *J* 8.2 Hz, C*H*-3′,5′), 7.15 (1H, s, C*H*-α), 6.88 (1H, dd, *J* 8.9, 2.1 Hz, C*H*-6), 6.58 (1H, ddd, *J* 8.9, 8.8, 2.1 Hz, C*H*-4), 3.59 (2H, s, 3-CH_2), 2.55 (3H, s, SCH_3) and 2.20 (3H, s, 2-CH_3); **^{13}C NMR** (100 MHz; $CDCl_3$) δ 175.3 (quat., carboxyl), 163.1 (quat., d, *J* 246.1 Hz, C-5), 146.2 (quat., d, *J* 8.4 Hz, C-8), 140.0 (quat., C-1), 139.2 (quat., C-4′), 138.8 (quat., C-2), 132.9 (quat., C-3), 130.2 (quat., C-1′), 130.0 (*C*H, C-α), 129.9 (*C*H × 2, C-2′,6′), 129.7 (quat., C-9), 125.9 (*C*H × 2, C-3′,5′), 123.8 (*C*H, d, *J* 8.9 Hz, C-7), 110.7 (*C*H, d, d, *J* 22.7 Hz, C-6), 105.7 (*C*H, d, *J* 24.3 Hz, C-4), 31.3 (*C*H$_2$, 3-CH$_2$), 15.4 (*C*H$_3$, SCH$_3$) and 10.6 (*C*H$_3$, 2-CH$_3$); **^{19}F {^{1}H}** (376 MHz; $CDCl_3$) -114.2 (C*F*); ***m/z*** (ES$^+$) 363 ([M+Na]$^+$, 100%); **HRMS** *m/z* (ES)$^+$ calcd. for $C_{20}H_{17}FNaO_2S$ [M+Na]$^+$ requires 363.0831, found 363.0839; **CHN** Anal. calcd. for $C_{20}H_{17}FO_2S$: C, 70.57; H, 5.03. Found C, 70.61; H, 5.11. The data were in agreement with the literature values.[308]

OR

Titanium (IV) chloride (1.0 M solution in CH_2Cl_2, 8.4 mmol, 8.4 mL) was added to a suspension of zinc dust (1.1 g, 16.8 mmol) in tetrahydrofuran (20 mL) cooled in an ice/acetone bath and the resulting suspension stirred for 10 min. A suspension of sulindac **112** (1.0 g, 2.8 mmol) in dichloromethane (20 mL) was added dropwise *via* syringe pump over 0.5 h and the resultant suspension stirred for 2 h, maintaining cooling. The suspension was filtered through a pad of Celite and the resulting solution was quenched with aq. HCl solution (3 N, 100 mL), the solution was extracted with dichloromethane (3 × 60 mL) and the organics combined and dried over Na_2SO_4, filtered and the solvent removed under reduced pressure to yield (*Z*)-2-(5-fluoro-2-methyl-1-(4-(methylthio)benzylidene)-1*H*-inden-3-yl)acetic acid, sulindac sulfide **115** (952 mg, 1.00 mmol, quant.) as a yellow solid, which was used without any further purification. The data were in agreement with the literature values and those reported previously.[308]

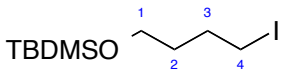
(*Z*)-2-(5-Fluoro-2-methyl-1-(4-(methylsulfonyl)benzylidene)-1*H*-inden-3-yl)acetic acid. (sulindac sulfone), 116[308]



Oxone® (3.08 g, 5.0 mmol) was added to a solution of sulindac **112** (356 mg, 1.0 mmol) in a mixture of tetrahydrofuran: MeOH: water. (3:1:1, 20 mL) and stirred for 1 hour at room temperature. The suspension was concentrated under reduced pressure to half of its original volume. Water (50 mL) was added and the precipitate collected by vacuum filtration to yield (*Z*)-2-(5-fluoro-2-methyl-1-(4-(methylsulfonyl)benzylidene)-1*H*-inden-3-yl)acetic acid, sulindac sulfone **116** (372 mg, 1.0 mmol, quant.) as a yellow solid, which was used without any further purification: **m.p.** 196–198 °C, [Lit.[308] 199-200 °C,]; $\boldsymbol{\nu_{max}}$ (thin film)/cm^{-1} 2078, 1720, 1493, 1400, 1255, 1147, 1048; **^{1}H NMR** (300 MHz; $CDCl_3$) δ 8.00 (2H, d, *J* 8.4 Hz, C*H*-3′,5′), 7.70 (2H, d, *J* 8.4 Hz, C*H*-2′,6′), 7.14 (1H, s, C*H*-α), 7.10 (1H, dd, *J* 5.1, 8.4 Hz, C*H*-7), 6.89 (1H, dd, *J* 8.8, 2.4 Hz, C*H*-6), 6.58 (1H, ddd, *J* 8.9, 8.8, 2.4 Hz, C*H*-4), 3.60 (2H, s, 3-

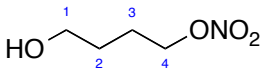
CH_2), 3.14 (3H, s, SO_2CH_3) and 2.21 (3H, s, 2-CH_3); **^{13}C NMR** (100 MHz; $CDCl_3$) δ 174.6 (quat., carboxyl), 162.3 (quat., d, *J* 247.0 Hz, C-5), 146.6 (quat., d, *J* 9.1 Hz, C-8), 142.5 (quat., C-4′), 142.2 (quat., C-1′), 139.9 (quat., C-1), 138.5 (quat., C-2), 131.6 (quat., C-3), 130.2 (*C*H × 2, C-2′,6′), 129.7 (quat., C-9), 129.2 (*C*H, C-α), 127.2 (*C*H × 2, C-3′,5′), 123.8 (*C*H, d, *J* 8.4 Hz, C-7), 111.1 (*C*H, d, *J* 22.6 Hz, C-6), 106.3 (*C*H, d, *J* 24.2 Hz, C-4), 44.5 (*C*H_3, SO_2CH_3), 31.2 (*C*H_2, 3-CH_2) and 10.5 (*C*H_3, 2-CH_3); **^{19}F {^{1}H}** (376 MHz; $CDCl_3$) δ -112.7 (C*F*); ***m/z*** (ES^+) 395 ($[M+Na]^+$, 100%); **HRMS** *m/z* (ES^+) calcd. for $C_{20}H_{17}FO_4NaS$ $[M+Na]^+$ requires 395.0729, found 395.0722; **CHN** Anal. calcd. for $C_{20}H_{17}FO_4S$: C, 64.50; H, 4.60. Found C, 64.59; H, 4.70. The data were in agreement with the literature values.[308]

tert-Butyl(4-iodobutoxy)dimethylsilane, 122[313]



THF (8.2 mL, 100 mmol) was added to dry acetonitrile (80 mL), followed by addition of sodium iodide (12.0 g, 80.0 mmol) and *tert*-butyldimethylsilyl chloride (6.03 g, 40.0 mmol) and the solution heated at 55 °C for 18 h. Water was added (150 mL) and the suspension extracted with petroleum ether/diethyl ether (9:1, 3 × 100 mL). The combined organic extracts were washed with saturated sodium hydrogen sulfite solution (100 mL) and brine (100 mL), dried over Na_2SO_4, filtered and the solvent removed under reduced pressure to give *tert*-butyl(4-iodobutoxy)dimethylsilane **122** (12.5 g, 40.0 mmol, quant.) as a colorless liquid, which was used without any further purification: **^{1}H NMR** (300 MHz; $CDCl_3$) δ 3.64 (2H, t, *J* 6.2 Hz, CH_2-1), 3.22 (2H, t, *J* 6.9 Hz, CH_2-4), 1.91 (2H, quint, *J* 6.9 Hz, CH_2-2), 1.66-1.51 (2H, m, CH_2-3), 0.89 (9H, s, CH_3 × 3, $SiC(CH_3)_3$) and 0.05 (6H, s, CH_3 × 3, $Si(CH_3)_2$); **^{13}C NMR** (100 MHz; $CDCl_3$) δ 62.4 (*C*H_2, C-1), 33.9 (*C*H_2, C-4), 30.6 (*C*H_2, C-2), 26.4 (*C*H_3 × 3, $SiC(CH_3)_3$), 18.7 (quat., $SiC(CH_3)_3$), 7.5 (*C*H_2, C-3) and -4.9 (*C*H_3 × 2, $Si(CH_3)_2$); **m/z** (ES^+) 337 ($[M+Na]^+$, 100%). The data were in agreement with the literature values.[313]

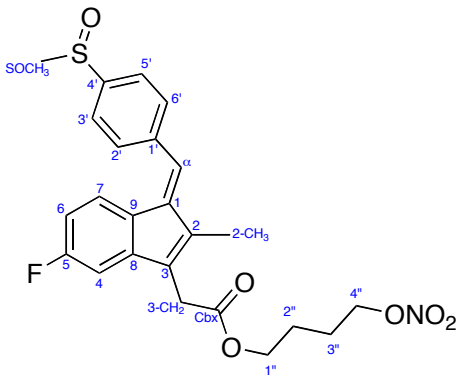
4-Nitrooxybutan-1-ol, 120[314]



Silver nitrate (1.3 g, 7.65 mmol) was added to a solution of *tert*-butyl(4-iodobutoxy)dimethylsilane **122** (1.2 g, 3.82 mmol) in dry acetonitrile (20 mL) at -10 °C in the absence of light. After 15 min, the solution was allowed to warm to room temperature and

stirred for a further hour. The suspension was filtered and water was added (2 mL) and the solution was stirred for a further hour. The solution was partitioned between ethyl acetate (40 mL) and water (30 mL), the organic layer was separated and dried over Na_2SO_4 and the solvent removed under reduced pressure to yield 4-nitrooxybutanol **120** (150 mg, 1.11 mmol, 29%) as a pale yellow oil, which was used without any further purification: **^{1}H NMR** (300 MHz; $CDCl_3$) δ 4.50 (2H, t, *J* 6.7 Hz, C*H*$_2$-4), 3.69 (2H, t, *J* 6.1 Hz, C*H*$_2$-1), 1.89-1.79 (2H, m, C*H*$_2$-3), 1.75 (1H, s, *br*, O*H*) and 1.72-1.62 (2H, m, C*H*$_2$-2); **^{13}C NMR** (100 MHz; $CDCl_3$) δ 73.1 (*C*H$_2$, C-4), 61.9 (*C*H$_2$, C-1), 28.6 (*C*H$_2$, C-3) and 23.1 (*C*H$_2$, C-2); **m/z** (ES^-) 118 ($[M-H]^-$, 100%). The data were in agreement with the literature values.[314]

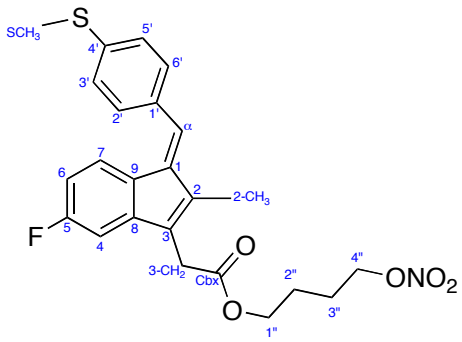
(*Z*)-4-(Nitrooxy)butyl 2-(5-fluoro-2-methyl-1-(4-(methylsulfinyl)benzylidene)-1*H*-inden-3-yl)acetate, 111



Following general procedure A with sulindac **112** (47 mg, 0.13 mmol) and 4-(nitrooxy)butanol **120** (15 mg, 0.13 mmol), purification by flash chromatography, eluting with dichloromethane and methanol (99:1), furnished *(Z)-4-(nitrooxy)butyl 2-(5-fluoro-2-methyl-1-(4-(methylsulfinyl)benzylidene)-1H-inden-3-yl)acetate* **111** (58 mg, 0.12 mmol, 91%) as a yellow solid: R_f 0.48 (95:5, CH_2Cl_2:MeOH, UV/cerium phosphomolybdate); **m.p**. 60-62 °C; **ν_{max}** (thin film)/cm^{-1} 1732, 1626, 1466, 1280, 1161, 1047, 967, 863, 820; **^{1}H NMR** (400 MHz; $CDCl_3$) δ 7.73 (2H, d, *J* 8.5 Hz, C*H*-3′,5′), 7.68 (2H, d, *J* 8.5 Hz, C*H*-2′,6′), 7.18 (1H, s, C*H*-α), 7.16 (1H, dd, *J* 5.4, *J* 8.5 Hz, C*H*-7), 6.88 (1H, dd, *J* 8.9, 2.9 Hz, C*H*-4), 6.58 (1H, ddd, *J* 8.9, 8.2, 2.9 Hz, C*H*-6), 4.40 (2H, t, *J* 5.3 Hz, C*H*$_2$-4″), 4.16 (2H, t, *J* 5.6 Hz, C*H*$_2$-1″), 3.58 (2H, s, 3-C*H*$_2$), 2.82 (3H, s, SOC*H*$_3$), 2.22 (3H, s, 2-C*H*$_3$) and 1.76-1.73 (4H, m, C*H*$_2$ × 2); **^{13}C NMR** (100 MHz; $CDCl_3$) δ 170.1 (quat., carboxyl), 162.9 (quat., d, *J* 244.9 Hz, C-5), 146.6 (quat., d, *J* 9.0 Hz, C-8), 145.6 (quat., C-4′), 141.6 (quat., C-1), 139.6 (quat., C-1′), 138.2 (quat., C-2), 131.7 (quat., C-3), 130.3 (*C*H × 2, C-2′,6′), 129.5 (quat., C-9), 128.4 (*C*H, C-α), 123.9 (*C*H × 2, C-3′,5′), 123.8 (*C*H, d, *J* 9.2 Hz, C-7), 110.9 (*C*H, d, *J* 22.5 Hz, C-6), 106.1 (*C*H, d, *J* 23.9 Hz, C-4), 72.5 (*C*H$_2$, C-4″), 64.2 (*C*H$_2$, C-1″), 43.9 (*C*H$_3$, SO*C*H$_3$), 31.8 (*C*H$_2$, 3-*C*H$_2$), 24.9 (*C*H$_2$, C-2″), 23.6 (*C*H$_2$,

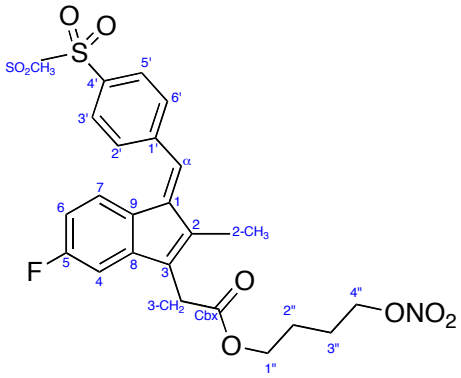
C-3′) and 10.5 (*C*H$_3$, 2-*C*H$_3$); **^{19}F {^{1}H} NMR** (376 MHz; CDCl$_3$) δ -113.4 (C*F*); ***m/z*** (ES$^+$) 473 ([M+Na]$^+$, 100%); **HRMS** *m/z* (ES$^+$) calcd. for $C_{24}H_{24}FNNaO_6S$ [M+Na]$^+$ requires 473.1308 found 473.1300; **CHN** Anal. calcd. for $C_{24}H_{24}FNO_6S$: C, 60.88; H, 5.11; N, 2.96. Found C, 60.94; H, 5.09; N, 3.00.

(*Z*)-4-(Nitrooxy)butyl 2-(5-fluoro-2-methyl-1-(4-(methylthio)benzylidene)-1*H*-inden-3-yl)acetate, 123



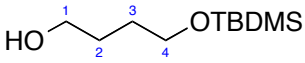
Following general procedure A with sulindac sulfide **115** (45 mg, 0.13 mmol) and 4-(nitrooxy)butanol **120** (15 mg, 0.13 mmol), purification by flash chromatography, eluting with dichloromethane and methanol (99:1), furnished *(Z)-4-(nitrooxy)butyl 2-(5-fluoro-2-methyl-1-(4-(methylthio)benzylidene)-1H-inden-3-yl)acetate* **123** (58 mg, 0.13 mmol, 95%) as a yellow solid: ***R$_f$*** 0.92 (95:5, CH_2Cl_2:MeOH, UV/cerium phosphomolybdate); **m.p.** 54-57 °C; **ν_{max}** (thin film)/cm^{-1} 1746, 1633, 1450, 1270, 1160, 1051, 870, 820; **^{1}H NMR** (400 MHz; $CDCl_3$) δ 7.45 (2H, d, *J* 8.3 Hz, C*H*-3′,5′), 7.38 (1H, dd, *J* 8.4 5.3 Hz, C*H*-7), 7.30 (2H, d, *J* 8.3 Hz, C*H*-2′,6′), 7.16 (1H, s, C*H*-α), 6.88 (1H, dd, *J* 9.0, 2.5 Hz, C*H*-4), 6.60 (1H, ddd, *J* 9.0, 8.9, 2.5 Hz, C*H*-6), 4.40 (2H, t, *J* 6.1 Hz, CH_2-4″), 3.63 (2H, t, *J* 5.9 Hz, CH_2-1″), 3.58 (2H, s, 3-CH_2), 2.56 (3H, s, SCH_3), 2.22 (3H, s, 2-CH_3) and 1.76-1.73 (4H, m, CH_2 × 2); **^{13}C NMR** (100 MHz; $CDCl_3$) δ 170.3 (quat., carboxyl), 163.0 (quat., d, *J* 247.5 Hz, C-5), 146.4 (quat., d, *J* 9.3 Hz, C-8), 140.0 (quat., C-1), 139.2 (quat., C-4′), 138.5 (quat., C-2), 132.9 (quat., C-3), 130.6 (*C*H, C-α), 130.1 (quat., C-1′), 129.9 (*C*H × 2, C-2′,6′), 129.8 (quat., C-9), 126.0 (*C*H × 2, C-3,5′), 123.8 (*C*H, d, *J* 8.8 Hz, C-7), 110.6 (*C*H, d, *J* 22.9 Hz, C-6), 105.7 (*C*H, d, *J* 23.7 Hz, C-4), 72.5 (*C*H$_2$, C-4″), 64.1 (*C*H$_2$, C-1″), 31.9 (*C*H$_2$, 3-*C*H$_2$), 25.0 (*C*H$_2$, C-3″), 25.1 (*C*H$_2$, C-2″), 15.4 (*C*H$_3$, S*C*H$_3$) and 10.6 (*C*H$_3$, 2-*C*H$_3$); **^{19}F {^{1}H} NMR** (376 MHz; $CDCl_3$) δ -114.4 (C*F*); ***m/z*** (ES$^+$) 457 ([M+Na]$^+$, 100%); **HRMS** *m/z* (ES$^+$) calcd. for $C_{24}H_{24}FNNaO_5S$ [M+Na]$^+$ requires 457.1359 found 457.1360; **CHN** Anal. calcd. for $C_{24}H_{24}FNO_5S$: C, 63.00; H, 5.29; N, 3.06. Found C, 63.04; H, 5.35; N, 3.09.

(*Z*)-4-(Nitrooxy)butyl 2-(5-fluoro-2-methyl-1-(4-(methylsulfonyl)benzylidene)-1*H*-inden-3-yl)acetate, 124



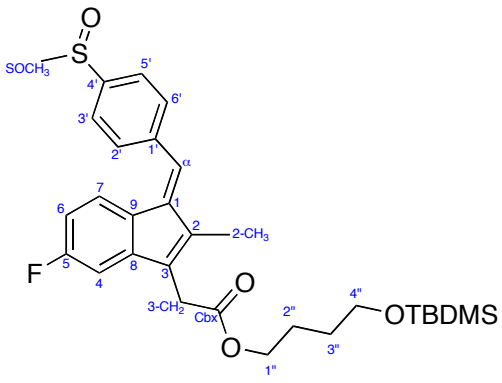
Following general procedure A with sulindac sulfone **116** (49 mg, 0.13 mmol) and 4-(nitrooxy)butanol **120** (15 mg, 0.13 mmol), purification by flash chromatography, eluting with dichloromethane and methanol (99:1), furnished *(Z)-4-(nitrooxy)butyl 2-(5-fluoro-2-methyl-1-(4-(methylsulfonyl)benzylidene)-1H-inden-3-yl)acetate* **124** (48 mg, 0.10 mmol, 73%) as a yellow solid: $\boldsymbol{R_f}$ 0.68 (95:5, CH_2Cl_2:MeOH, UV/cerium phosphomolybdate); **m.p.** 65-69 °C; $\boldsymbol{\nu_{max}}$ (thin film)/cm^{-1} 1751, 1620, 1454, 1271, 1174, 105, 892, 815; **^{1}H NMR** (400 MHz; $CDCl_3$) δ 8.01 (2H, d, *J* 8.4 Hz, C*H*-3′,5′), 7.70 (2H, d, *J* 8.4 Hz, C*H*-2′,6′), 7.14 (1H, s, C*H*-α), 7.10 (1H, dd, *J* 8.4, 5.4 Hz, C*H*), 6.87 (1H, dd, *J* 8.8, 2.4 Hz, C*H*-4), 6.58 (1H, ddd, *J* 8.8, 8.8, 2.4 Hz, C*H*-6), 4.40 (2H, t, *J* 6.1 Hz, CH_2-4″), 4.15 (2H, q, *J* 6.1 Hz, CH_2-1″), 3.57 (2H, s, 3-CH_2), 3.14 (3H, s, SO_2CH_3), 2.21 (3H, s, 2-CH_3) and 1.76-1.73 (4H, m, CH_2 × 2); **^{13}C NMR** (100 MHz; $CDCl_3$) δ 170.0 (quat., carboxyl), 163.4 (quat., d, *J* 247.2 Hz, C-5), 146.7 (quat., d, *J* 8.4 Hz, C-8), 142.5 (quat., C-4′), 142.3 (quat., C-1′), 139.9 (quat., C-1), 138.1 (quat., C-2), 132.2 (quat., C-3), 130.2 (*C*H × 2, C-2′,6′), 129.3 (quat., C-9), 127.6 (*C*H × 2, C-3,5′), 127.4 (*C*H, C-α), 123.8 (*C*H, d, *J* 9.8 Hz, C-7), 111.0 (*C*H, d, *J* 22.6 Hz, C-6), 106.3 (*C*H, d, *J* 24.9 Hz, C-4), 72.5 (*C*H_2, C-4″), 64.2 (*C*H_2, C-1″), 44.5 (*C*H_3, SO_2CH_3), 31.8 (*C*H_2, 3-*C*H_2), 24.9 (*C*H_2, C-2″), 23.6 (*C*H_2, C-3″) and 10.5 (*C*H_3, 2-*C*H_3); **^{19}F {^{1}H} NMR** (376 MHz; $CDCl_3$) δ -112.9 (C*F*); ***m/z*** (ES^+) 489 ($[M+Na]^+$, 100%); **HRMS** *m/z* (ES^+) calcd. for $C_{24}H_{24}FNNaO_7S$ $[M+Na]^+$ requires 489.1258 found 489.1256. **CHN** Anal. calcd. for $C_{24}H_{24}FNO_7S$: C, 59.89; H, 4.29; N, 2.86. Found C, 59.91; H, 4.35; N, 3.00.

4-((*tert*-Butyldimethylsilyl)oxy)butan-1-ol, 126[316]



Triethylamine (7.26 g, 10.0 mL, 73.4 mmol) and TBDMSCl (7.38 g, 4.89 mmol) were added to a solution of 1,4-butanediol **125** (22.4 g, 22.0 mL, 245 mmol) in CH_2Cl_2 (300 mL) and the resultant solution stirred overnight at room temperature. The solution was poured into brine (100 mL) and the organic layer was separated and washed with distilled water (100 mL) and brine (75 mL). The organic layer was dried over Na_2SO_4, filtered and the solvent removed under reduced pressure to give a pale yellow oil. This oil was purified by silica gel chromatography, eluting with petroleum ether and ethyl acetate (99:1), to afford 4-((*tert*-butyldimethylsilyl)oxy)butan-1-ol **126** (6.80 g, 3.32 mmol, 68%) as a colourless liquid: $\boldsymbol{R_f}$ 0.30 (70:30, PE:EtOAc, cerium phosphomolybdate); **^{1}H NMR** (400 MHz; $CDCl_3$) δ 3.64-3.58 (4H, m, CH_2-1,4), 2.68 (1H, t, *J* 5.8, O*H*), 1.65-1.60 (4H, m, CH_2-2,3), 0.90 (9H, s, SiC(CH_3)$_3$) and 0.05 (6H, s, Si(CH_3)$_2$); **^{13}C NMR** (100 MHz; $CDCl_3$) δ 63.2 (*C*H_2), 62.7 (*C*H_2), 30.0 (*C*H_2), 29.7 (*C*H_2), 25.9 (*C*H_3 × 3, SiC(CH_3)$_3$), 18.3 (quat., SiC(CH_3)$_3$) and -5.5 (*C*H_3 × 2, Si(*C*H_3)$_2$); ***m/z*** (ES$^+$) 227 ([M+Na]$^+$, 100%). The data were in agreement with the literature values.[316]

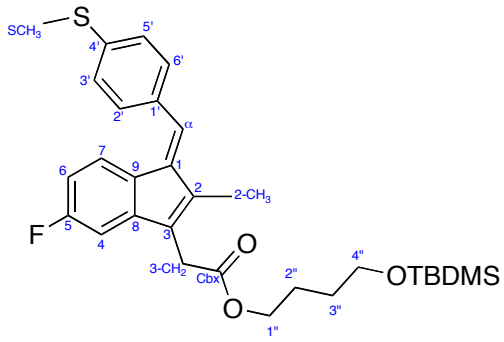
(*Z*)-4-((*tert*-Butyldimethylsilyl)oxy)butyl 2-(5-fluoro-2-methyl-1-(4-(methylsulfinyl)-benzylidene)-1*H*-inden-3-yl)acetate, 127



Following general procedure A with sulindac **112** (172 mg, 0.49 mmol) and 4-((*tert*-butyldimethylsilyl)oxy)butan-1-ol **126** (100 mg, 0.49 mmol), purification by flash chromatography, eluting with dichloromethane and methanol (99:1), furnished *(Z)-4-((tert-butyldimethylsilyl)oxy)butyl-2-(5-fluoro-2-methyl-1-(4-(methylsulfinyl)-benzylidene)-1H-inden-3-yl)acetate* **127** (226 mg, 0.42 mmol, 85%) as a yellow solid: $\boldsymbol{R_f}$ 0.56 (95:5, CH_2Cl_2:MeOH, UV/cerium phosphomolybdate); **m.p.** 59-61 °C; $\boldsymbol{\nu_{max}}$ (thin film)/cm^{-1} 2869, 1745, 1601, 1591,

1491, 1466, 1317, 1256, 1185, 1092, 810; **^{1}H NMR** (400 MHz; $CDCl_3$) δ 7.72 (2H, d, *J* 8.4 Hz, C*H*-3′,5′), 7.67 (2H, d, *J* 8.4 Hz, C*H*-2′,6′), 7.15 (1H, s, C*H*-α), 7.14 (1H, dd, *J* 8.4, 5.0 Hz, C*H*-7), 6.89 (1H, dd, *J* 8.9, 2.4 Hz, C*H*-4), 6.55 (1H, ddd, *J* 8.9, 8.9, 2.4 Hz, C*H*-6), 4.13 (2H, t, *J* 6.6 Hz, CH_2-1″, 3.61 (2H, t, *J* 6.2 Hz, CH_2-4″), 3.56 (2H, s, 3-CH_2), 2.81 (3H, s, $SOCH_3$), 2.21 (3H, s, 2-CH_3), 1.71-1.65 (2H, m, CH_2-2″), 1.56-1.50 (2H, m, CH_2-3″), 0.89 (9H, s, $SiC(CH_3)_3$) and 0.04 (6H, s, $Si(CH_3)_2$); **^{13}C NMR** (100 MHz; $CDCl_3$) δ 170.3 (quat., carboxyl), 163.4 (quat., d, *J* 248.7 Hz, C-5), 146.8 (quat., d, *J* 8.7 Hz, C-8), 145.5 (quat., C-4′), 141.7 (quat., C-1), 139.7 (quat., C-1′), 138.1 (quat., C-2), 132.0 (quat., C-3), 130.3 (*C*H × 2, C-2′,6′), 129.5 (quat., C-9), 128.1 (*C*H, C-α), 123.8 (*C*H × 2, C-3,5′), 123.6 (*C*H, d, *J* 8.8 Hz, C-7), 110.7 (*C*H, d, *J* 22.7 Hz, C-6), 106.2 (*C*H, d, *J* 23.9 Hz, C-4), 65.1 (CH_2, C-1″), 62.5 (CH_2, C-4″), 43.9 (CH_3, $SOCH_3$), 31.9 (CH_2, 3-CH_2), 29.1 (CH_2, C-2″), 25.9 (CH_3 × 3, $SiC(CH_3)_3$), 25.3 (CH_2, C-3″), 18.3 (quat., $SiC(CH_3)_3$), 10.6 (CH_3, 2-CH_3) and -5.3 (CH_3 × 2, $Si(CH_3)_2$); **^{19}F {^{1}H} NMR** (376 MHz; $CDCl_3$) δ -113.4 (C*F*); ***m/z*** (ES^+) 565 ($[M+Na]^+$, 100%); **HRMS** *m/z* (ES^+) calcd. for $C_{30}H_{39}FNaO_4SSi$ $[M+Na]^+$ requires 565.2220, found 565.2219.

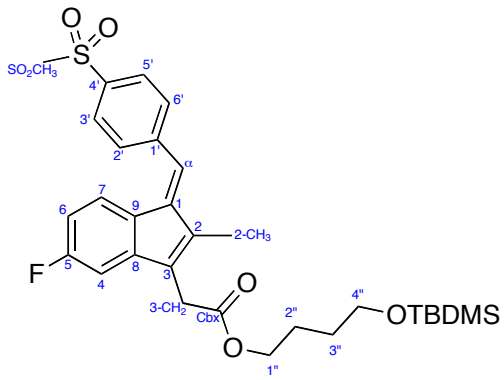
(*Z*)-4-((*tert*-Butyldimethylsilyl)oxy)butyl 2-(5-fluoro-2-methyl-1-(4-(methylthio)-benzylidene)-1*H*-inden-3-yl)acetate, 128



Following general procedure A with sulindac sulfide **115** (163 mg, 0.49 mmol) and 4-((*tert*-butyldimethylsilyl)oxy)butan-1-ol **126** (100 mg, 0.49 mmol), purification by flash chromatography, eluting with dichloromethane, furnished *(Z)-4-((tert-butyldimethylsilyl)oxy)butyl 2-(5-fluoro-2-methyl-1-(4-(methylthio)benzylidene)-1H-inden-3-yl)acetate* **128** (253 mg, 0.48 mmol, 98%) as a yellow solid: R_f 0.90 (95:5, CH_2Cl_2:MeOH, UV/cerium phosphomolybdate); **m.p.** 64-65 °C; ν_{max} (thin film)/cm^{-1} 2954, 2927, 1734, 1622, 1602, 1491, 1467, 1317, 1255, 836, 810; **^{1}H NMR** (400 MHz; $CDCl_3$) δ 7.45 (2H, d, *J* 8.4 Hz, C*H*-3′,5′), 7.36 (1H, dd, *J* 8.4, 5.2 Hz, C*H*-7), 7.30 (2H, d, *J* 8.4 Hz, C*H*-2′,6′), 7.14 (1H, s, C*H*-α), 6.90 (1H, dd, *J* 9.0, 2.4 Hz, C*H*-4), 6.58 (1H, ddd, *J* 9.0, 8.8, 2.4 Hz, C*H*-6), 4.13 (2H, t, *J* 6.5 Hz, CH_2-1″), 3.61 (2H, t, *J* 6.2 Hz, CH_2-4″), 3.56 (2H, s, 3-CH_2), 2.56 (3H, s, SCH_3), 2.21

(3H, s, 2-CH_3), 1.74-1.65 (2H, m, CH_2-2″), 1.58-1.49 (2H, m, CH_2-3″), 0.89 (9H, s, SiC(CH_3)$_3$) and 0.04 (6H, s, Si(CH_3)$_2$); **^{13}C NMR** (100 MHz; $CDCl_3$) δ 170.4 (quat., carboxyl), 163.4 (quat., d, *J* 245.5 Hz, C-5), 146.5 (quat., d, *J* 8.7 Hz, C-8), 140.2 (quat., C-1), 139.1 (quat., C-4′), 138.6 (quat., C-2), 133.1 (quat., C-3), 130.9 (quat., C-1′), 129.9 (*C*H × 2, C-2′,6′), 129.8 (quat., C-9), 131.0 (*C*H, C-α), 126.0 (*C*H × 2, C-3,5′), 123.6 (*C*H, d, *J* 8.7 Hz, C-7), 110.5 (*C*H, d, *J* 22.5 Hz, C-6), 105.8 (*C*H, d, *J* 23.9 Hz, C-4), 65.0 (*C*H_2, C-1″), 62.5 (*C*H_2, C-4″), 31.9 (*C*H_2, 3-*C*H_2), 29.1 (*C*H_2, C-2″), 25.9 (*C*H_3 × 3, SiC(*C*H_3)$_3$), 25.2 (*C*H_2, C-3″), 18.3 (quat., Si*C*(CH$_3$)$_3$), 15.4 (*C*H_3, S*C*H$_3$), 10.6 (*C*H_3, 2-*C*H_3) and -5.3 (*C*H_3 × 2, Si(*C*H_3)$_2$); **^{19}F {^{1}H} NMR** (376 MHz; $CDCl_3$) δ -114.4 (C*F*); ***m/z*** (ES^+) 549 ($[M+Na]^+$, 100%); **HRMS** *m/z* (ES^+) calcd. for $C_{30}H_{39}FNaO_3SSi$ $[M+Na]^+$ requires 549.2271, found 549.2269.

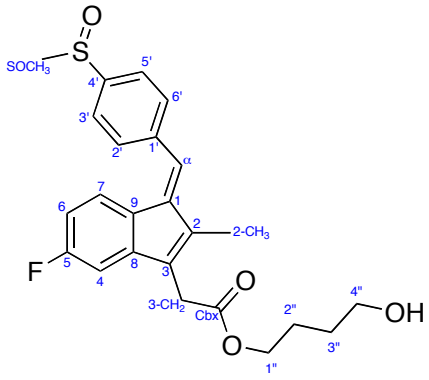
(*Z*)-4-((*tert*-Butyldimethylsilyl)oxy)butyl 2-(5-fluoro-2-methyl-1-(4-(methylsulfonyl)-benzylidene)-1*H*-inden-3-yl)acetate, 129



Following general procedure A with sulindac sulfone **116** (182 mg, 0.49 mmol) and 4-((*tert*-butyldimethylsilyl)oxy)butan-1-ol **126** (100 mg, 0.49 mmol), purification by flash chromatography, eluting with dichloromethane and methanol (99:1), furnished *2-(5-fluoro-2-methyl-1-(4-(methylsulfonyl)benzylidene)-1H-inden-3-yl)acetate* **129** (244 mg, 0.44 mmol, 89%) as a yellow solid: R_f 0.69 (95:5, CH_2Cl_2:MeOH, UV/cerium phosphomolybdate); **m.p.** 67-68 °C; ν_{max} (thin film)/cm^{-1} 2928, 2858, 1734, 1604, 1592, 1470, 1389, 1311,1255, 1150, 973, 833, 770; **^{1}H NMR** (400 MHz; $CDCl_3$) δ 8.01 (2H, d, *J* 8.4 Hz, C*H*-3′,5′), 7.70 (2H, d, *J* 8.4 Hz, C*H*-2′,6′), 7.12 (1H, s, C*H*-α), 7.10 (1H, dd, *J* 8.3, 5.1 Hz, C*H*-7), 6.89 (1H, dd, *J* 8.9, 2.4 Hz, C*H*-4), 6.57 (1H, ddd, *J* 17.4, 8.9, 2.4 Hz, C*H*-6), 4.14 (2H, t, *J* 6.6 Hz, CH_2-1″), 3.61 (2H, t, *J* 6.1 Hz, CH_2-4″), 3.56 (2H, s, 3-CH_2), 3.14 (3H, s, SO_2CH_3), 2.21 (3H, s, 2-CH_3), 1.75-1.65 (2H, m, CH_2-2″), 1.59-1.50 (2H, m, CH_2-3″), 0.89 (9H, s, SiC(*C*H_3)$_3$) and 0.04 (6H, s, Si(*C*H_3)$_2$); **^{13}C NMR** (100 MHz; $CDCl_3$) δ 170.2 (quat., carboxyl), 163.7 (quat., d, *J* 246.7 Hz, C-5), 146.9 (quat., d, *J* 8.8 Hz, C-8), 142.6 (quat., C-4′), 141.7 (quat., C-1′), 139.9 (quat., C-1), 138.0 (quat., C-2), 132.6 (quat., C-3), 130.2 (*C*H × 2, C-2′,6′), 129.3 (quat., C-9), 127.6 (*C*H × 2, C-3′,5′),

127.0 (*C*H, C-α), 123.7 (*C*H, d, *J* 9.1 Hz, C-7), 110.9 (*C*H, d, *J* 22.7 Hz, C-6), 106.4 (*C*H, d, *J* 24.2 Hz, C-4), 65.2 ($C H_2$, C-1″), 62.5 ($C H_2$, C-4″), 44.5 ($C H_3$, SO_2CH_3), 31.9 ($C H_2$, 3-$C H_2$), 29.1 ($C H_2$, C-2″), 25.9 ($C H_3$ × 3, SiC($C H_3$)$_3$), 25.3 ($C H_2$, C-3″), 18.3 (quat., Si*C*(CH_3)$_3$), 10.5 ($C H_3$, 2-$C H_3$) and -5.3 ($C H_3$ × 2, Si($C H_3$)$_2$); **^{19}F {^{1}H} NMR** (376 MHz; $CDCl_3$) δ -112.9 (C*F*); ***m/z*** (ES^+) 581 ($[M+Na]^+$, 100%); **HRMS** *m/z* (ES^+) calcd. for $C_{30}H_{39}FNaO_5SSi$ $[M+Na]^+$ requires 581.2169, found 581.2170.

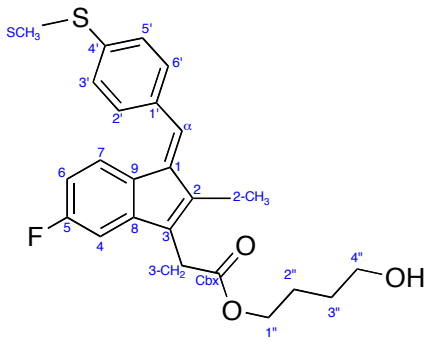
(*Z*)-4-Hydroxybutyl 2-(5-fluoro-2-methyl-1-(4-(methylsulfinyl)benzylidene)-1*H*-inden-3-yl)acetate, 113



10-Camphorsulfonic acid (2 mg, 0.01 mmol) was added to a solution of sulindac TBDMS ester **127** (50 mg, 0.09 mmol) in CH_2Cl_2:MeOH (1:1, 4 mL) and the solution stirred for 3 h. Water (5 mL) was added and the solution extracted with CH_2Cl_2 (4 mL). The organic layer was separated, washed with brine (3 mL), dried over anhydrous Na_2SO_4, filtered and the solvent removed under reduced pressure to give the crude residue. Purification by silica gel chromatography, eluting with dichloromethane and methanol (99:1), to give *(Z)-4-hydroxybutyl 2-(5-fluoro-2-methyl-1-(4-(methylsulfinyl)benzylidene)-1H-inden-3-yl)acetate* **113** (32 mg, 0.06 mmol, 63%) as a yellow solid: R_f 0.38 (95:5, CH_2Cl_2:MeOH, UV/cerium phosphomolybdate); **m.p.** 72-74 °C; 3402, 2941, 1730, 1622, 1601, 1591, 1491, 1466, 1437, 1257, 1092, 811; **^{1}H NMR** (400 MHz; $CDCl_3$) δ 7.72 (2H, d, *J* 8.5 Hz, C*H*-3′,5′), 7.66 (2H, d, *J* 8.5 Hz, C*H*-2′,6′), 7.16 (1H, s, C*H*-α), 7.15 (1H, dd, *J* 8.3, 5.2 Hz, C*H*-7), 6.89 (1H, dd, *J* 9.0, 2.4 Hz, C*H*-4), 6.55 (1H, ddd, *J* 9.0, 8.9, 2.4 Hz, C*H*-6), 4.15 (2H, t, *J* 6.4 Hz, CH_2-1″), 3.63 (2H, q, *J* 6.1 Hz, CH_2-4″), 3.56 (2H, s, 3-CH_2), 2.81 (3H, s, SOCH_3), 2.21 (3H, s, 2-CH_3), 1.77-1.68 (2H, m, CH_2-2″), 1.63-1.54 (2H, m, CH_2-3″) and 1.25 (1H, t, *J* 6.1, O*H*); **^{13}C NMR** (100 MHz; $CDCl_3$) δ 170.3 (quat., carboxyl), 163.2 (quat., d, *J* 245.0 Hz, C-5), 146.7 (quat., d, *J* 8.3 Hz, C-8), 145.5 (quat., C-4′), 141.7 (quat., C-1), 139.7 (quat., C-1′), 138.2 (quat., C-2), 131.9 (quat., C-3), 130.3 (*C*H × 2, C-2′,6′), 129.5 (quat., C-9), 128.2 (*C*H, C-α), 123.8 (*C*H × 2, C-3,5′), 123.7 (*C*H, d, *J* 9.2 Hz, C-7), 110.8 (*C*H, d, *J* 23.3 Hz, C-6), 106.2 (*C*H, d, *J* 23.3 Hz, C-4), 65.0 ($C H_2$, C-1″), 62.3

(CH_2, C-4″), 43.9 (CH_3, SOCH_3), 31.9 (CH_2, 3-CH_2), 29.1 (CH_2, C-2″), 25.1 (CH_2, C-3″) and 10.6 (CH_3, 2-CH_3); **^{19}F {^{1}H} NMR** (376 MHz; $CDCl_3$) δ -113.4 (C*F*); ***m/z*** (ES^+) 451 ($[M+Na]^+$, 100%); **HRMS** *m/z* (ES^+) calcd. for $C_{24}H_{25}FNaO_4S$ $[M+Na]^+$ requires 451.1355 found 451.1352; **CHN** Anal. calcd. for $C_{24}H_{25}FO_4S$: C, 67.27; H, 5.88. Found C, 67.30; H, 5.92.

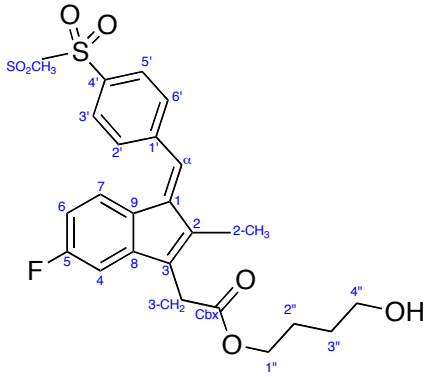
(*Z*)-4-Hydroxybutyl 2-(5-fluoro-2-methyl-1-(4-(methylthio)benzylidene)-1*H*-inden-3-yl)acetate, 130



10-Camphorsulfonic acid (2 mg, 0.01 mmol) was added to a solution of sulindac TBDMS ester **128** (50 mg, 0.10 mmol) in CH_2Cl_2:MeOH (1:1, 4 mL) and the solution stirred for 3 h. Water (5 mL) was added and the solution was extracted with CH_2Cl_2 (4 mL). The organic layer was separated and washed with brine (3 mL), dried over anhydrous Na_2SO_4, filtered and the solvent removed under reduced pressure to give the crude residue, which was purified by silica gel chromatography eluting with dichloromethane and methanol (99:1), to give *(Z)-4-hydroxybutyl 2-(5-fluoro-2-methyl-1-(4-(methylthio)benzylidene)-1H-inden-3-yl)acetate* **130** (32 mg, 0.08 mmol, 82%) as a yellow solid: ***R*$_f$** 0.12 (CH_2Cl_2); **m.p.** 59-60 °C; **ν_{max}** (thin film)/cm^{-1} 3400, 2928, 1730, 1603, 1591, 1467, 1310, 1150, 957, 855, 770; **^{1}H NMR** (400 MHz; $CDCl_3$) δ 7.45 (2H, d, *J* 8.1 Hz, C*H*-3′,5′), 7.37 (1H, dd, *J* 8.4, 5.2 Hz, C*H*-7), 7.30 (2H, d, *J* 8.1 Hz, C*H*-2′,6′), 7.15 (1H, s, C*H*-α), 6.90 (1H, dd, *J* 9.0, 2.4 Hz, C*H*-4), 6.59 (1H, ddd, *J* 9.0, 8.9, 2.4 Hz, C*H*-6), 4.15 (2H, t, *J* 6.5 Hz, CH_2-1″), 3.63 (2H, q, *J* 6.2 Hz, CH_2-4″), 3.57 (2H, s, 3-CH_2), 2.56 (3H, s, SCH_3), 2.22 (3H, s, 2-CH_3), 1.77-1.67 (2H, m, CH_2-2″), 1.62-1.53 (2H, m, CH_2-3″) and 1.27 (1H, t, *J* 6.1, O*H*). **^{13}C NMR** (100 MHz; $CDCl_3$) δ 170.4 (quat., carboxyl), 163.1 (quat., d, *J* 245.3 Hz, C-5), 146.5 (quat., d, *J* 8.1 Hz, C-8), 140.1 (quat., C-1), 139.2 (quat., C-4′), 138.4 (quat., C-2), 133.0 (quat., C-3), 131.9 (*C*H, C-α), 130.8 (quat., C-1′), 129.9 (*C*H × 2, C-2′,6′), 129.8 (quat., C-9), 126.0 (*C*H × 2, C-3,5′), 123.7 (*C*H, d, *J* 9.4 Hz, C-7), 110.5 (*C*H, d, *J* 22.3 Hz, C-6), 105.8 (*C*H, d, *J* 24.0 Hz, C-4), 64.9 (CH_2, C-1″), 62.3 (CH_2, C-4″), 31.9 (CH_2, 3-CH_2), 29.1 (CH_2, C-2″), 25.1 (CH_2, C-3″), 15.4 (CH_3, SCH_3) and 10.6 (CH_3, 2-CH_3); **^{19}F {^{1}H} NMR** (376 MHz; $CDCl_3$) δ -114.4 (C*F*); ***m/z*** (ES^+) 435 ($[M+Na]^+$, 100%); **HRMS** *m/z* (ES^+)

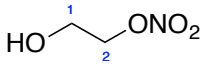
calcd. for $C_{24}H_{25}FNaO_3S$ $[M+Na]^+$ requires 435.1406 found 435.1400; **CHN** Anal. calcd. for $C_{24}H_{25}FO_3S$: C, 69.88; H, 6.11. Found C 69.93; H, 6.18.

(*Z*)-4-Hydroxybutyl 2-(5-fluoro-2-methyl-1-(4-(methylsulfonyl)benzylidene)-1*H*-inden-3-yl)acetate, 131



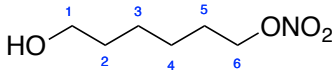
10-Camphorsulfonic acid (2 mg, 0.010 mmol) was added to a solution of sulindac TBDMS ester **129** (50 mg, 0.09 mmol) in CH_2Cl_2:MeOH (1:1, 4 mL) and the solution stirred for 3 h. Water (5 mL) was added and the solution was extracted with CH_2Cl_2 (4 mL). The organic layer was separated and washed with brine (3 mL), dried over anhydrous Na_2SO_4, filtered and the solvent removed under reduced pressure to give the crude residue, which was purified by silica gel chromatography, eluting with dichloromethane and methanol (99:1), to give *(Z)-4-hydroxybutyl 2-(5-fluoro-2-methyl-1-(4-(methylsulfonyl)benzylidene)-1H-inden-3-yl)acetate* **131** (30 mg, 0.07 mmol, 75%) as a yellow solid: R_f 0.32 (95:5, CH_2Cl_2:MeOH, UV/cerium phosphomolybdate); **m.p.** 81-82 °C; ν_{max} (thin film)/cm^{-1} 3345, 3063, 2938, 1736, 1606, 1592, 1468, 1299, 1181, 967; ^{1}**H NMR** (400 MHz; $CDCl_3$) δ 8.01 (2H, d, *J* 8.4 Hz, C*H*-3′,5′), 7.71 (2H, d, *J* 8.4 Hz, C*H*-2′,6′), 7.13 (1H, s, C*H*-α), 7.10 (1H, dd, *J* 8.4, 5.1 Hz, C*H*-7), 6.90 (1H, dd, *J* 8.8, 2.3 Hz, C*H*-4), 6.58 (1H, ddd, *J* 8.9, 8.8, 2.3 Hz, C*H*-6), 4.16 (2H, t, *J* 6.5 Hz, CH_2-1″), 3.65 (2H, q, *J* 6.1 Hz, CH_2-4″), 3.57 (2H, s, 3-CH_2), 3.14 (3H, s, SO_2CH_3), 2.22 (3H, s, 2-CH_3), 1.78-1.69 (2H, m, CH_2-2″) and 1.63-1.54 (2H, m, CH_2-3″); ^{13}**C NMR** (100 MHz; $CDCl_3$) δ 170.1 (quat., carboxyl), 163.4 (quat., d, *J* 245.0 Hz, C-5), 146.8 (quat., d, *J* 8.1 Hz, C-8), 142.5 (quat., C-4′), 141.4 (quat., C-1′), 139.9 (quat., C-1), 138.0 (quat., C-2), 132.4 (quat., C-3), 130.2 (*C*H × 2, C-2′,6′), 129.3 (quat., C-9), 127.6 (*C*H × 2, C-3,5′), 127.2 (*C*H, C-α), 123.7 (*C*H, d, *J* 9.8 Hz, C-7), 110.9 (*C*H, d, *J* 22.4 Hz, C-6), 106.4 (*C*H, d, *J* 22.4 Hz, C-4), 65.0 (CH_2, C-1″), 62.3 (CH_2, C-4″), 44.5 (CH_3, SO_2CH_3), 31.9 (CH_2, 3-CH_2), 29.1 (CH_2, C-2″), 25.1 (CH_2, C-3″) and 10.5 (CH_3, 2-CH_3); ^{19}**F {^{1}H} NMR** (376 MHz; $CDCl_3$) δ -112.9 (C*F*); ***m/z*** (ES^+) 467 ($[M+Na]^+$, 100%); **HRMS** *m/z* (ES^+) calcd. for $C_{24}H_{25}FNaO_5S$ $[M+Na]^+$ requires 467.1304 found 467.1309; **CHN** Anal. calcd. for $C_{24}H_{25}FO_5S$: C, 64.85; H, 5.67. Found C, 64.90; H, 5.75.

2-(Nitrooxy)ethanol, 136[318]



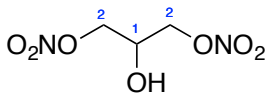
Silver nitrate (2.07 g, 13.2 mmol) was added to a solution of 2-bromoethanol **132** (500 mg, 4.00 mmol), in CH_3CN (15 mL) and stirred for 8 h under reflux. The mixture was poured onto brine (20 mL), and the precipitate was filtered. The filtrate was extracted with diethyl ether (5 × 20 mL), dried over $MgSO_4$, filtered and the solvent was removed under reduced pressure to yield 2-nitrooxyethanol **136** (364 mg, 3.40 mmol mmol, 85%) as a colourless oil, which was used without any further purification: **^{1}H NMR** (400 MHz; $CDCl_3$) δ 4.59 (2H, t, *J* 4.6 Hz, C*H*$_2$-2), 3.92 (2H, t, *J* 4.6 Hz, C*H*$_2$-1); **^{13}C NMR** (100 MHz; $CDCl_3$) δ 74.3 (*C*H2, C-1), 59.2 (*C*H$_2$, C-2); ***m/z*** (ES^+) 130 ($[M+Na]^+$, 100%). The data were in agreement with the literature values.[318]

6-(Nitrooxy)hexan-1-ol, 137[319]



Silver nitrate (698 mg, 4.11 mmol) was added to a solution of 6-bromohexan-1-ol **133** (500 mg, 2.76 mmol) in CH_3CN (15 mL) and stirred for 8 h under reflux. The mixture was poured onto brine (20 mL), and the precipitate was filtered. The filtrate was extracted with diethyl ether (5 × 20 mL), and dried over $MgSO_4$, filtered and the solvent was removed under reduced pressure to yield 6-(nitrooxy)hexan-1-ol **137** (405 mg, 2.48 mmol, 90%) as a clear pale yellow oil, which was used without any further purification: ***R$_f$*** 0.29 (30:70 EtOAc:PE, $KMnO_4$); **^{1}H NMR** (400 MHz; $CDCl_3$) δ 4.45 (2H, t, *J* 6.7 Hz, C*H*$_2$-6), 3.65 (2H, t, *J* 6.5 Hz, C*H*$_2$-1), 1.70-1.78 (2H, m, C*H*$_2$-5), 1.54-1.62 (2H, m, C*H*$_2$-2), 1.38-1.48 (5H, m, 2 × C*H*$_2$-3, 4, O*H*); **^{13}C NMR** (100 MHz;$CDCl_3$) δ 73.4 (*C*H$_2$, C-6), 62.6 (*C*H$_2$, C-1), 32.5 (*C*H$_2$, C-2), 26.8 (*C*H$_2$, C-5), 25.6, 25.4 (*C*H$_2$ × 2, C-3, 4); ***m/z*** (ES^+) 164 ($[M+H]^+$, 100%). The data were in agreement with the literature values.[319]

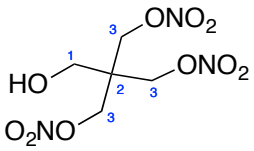
1,3-Di(nitrooxy)-2-propanol, 138[127]



Silver nitrate (1.80 g, 10.7 mmol) was added to a solution of 1,3-dibromo-2-propanol **134** (500 mg, 2.30 mmol) in CH_3CN (15 mL) and stirred for 8 h under reflux. The mixture was poured

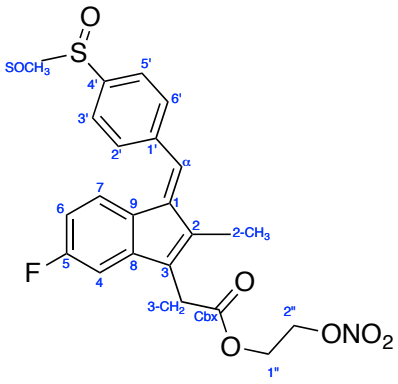
onto brine (20 mL), and the precipitate was filtered. The filtrate was extracted with diethyl ether (5 × 20 mL), dried over $MgSO_4$, filtered and the solvent was removed under reduced pressure to yield 1,3-di(nitrooxy)-2-propanol **138** (367 mg, 2.02 mmol, 88%) as a pale yellow oil, which was used without any further purification: R_f 0.50 (30:70 EtOAc:PE, $KMnO_4$); **^{1}H NMR** (400 MHz; $CDCl_3$) δ 4.58 (2H, dd, *J* 11.7, 4.5 Hz, 2 × CH_AH$_B$-2), 4.51 (2H, dd, *J* 11.7, 6.0 Hz, 2 × CH$_A$$H_B$-2), 4.23-4.29 (1H, m, C*H*-2); **^{13}C NMR** (100 MHz; $CDCl_3$) δ 72.6 (*C*H$_2$ × 2, C-2), 65.3 (*C*H, C-1); ***m/z*** (ES$^+$) 205 ([M+H]$^+$, 100%). The data were in agreement with the literature values.[127]

3-Nitrooxy-2,2-*bis*(nitrooxymethyl)propan-1-ol, 139



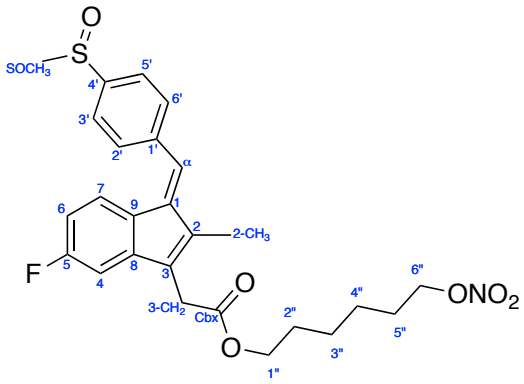
Silver nitrate (8.50 g, 50 mmol) was added to a solution of pentaerythritol tribromide **135** (1.00 g, 3.10 mmol), in CH_3CN (25 mL) and stirred at room temperature for 7 days. The solution was poured onto brine (25 mL) and the solid was removed by filtration. The filtrate was extracted with diethyl ether (6 × 20 mL) and washed with water (50 mL). The organic layer was dried over $MgSO_4$, filtered and the solvent was removed under reduced pressure. The residue was purified by flash column chromatography, eluting with ethyl acetate and hexane (5:95 to 20:90) to furnish 3-nitro-2,2-*bis*(nitromethyl)propan-1-ol **139** (220 mg, 0.80 mmol, 26%) as a colourless oil: R_f 0.67 (30:70 EtOAc:PE, $KMnO_4$); **^{1}H NMR** (400 MHz; $CDCl_3$) δ 4.57 (6H, s, 3 × CH_2-3), 3.75 (2H, d, *J* 4.8 Hz, CH_2-1), 2.45-2.53 (1H, m, O*H*); **^{13}C NMR** (100 MHz; $CDCl_3$) δ 69.7 (*C*H$_2$ × 3, C-3), 60.3 (*C*H$_2$, C-1), 43.4 (quat., C-2); ***m/z*** (ES$^+$) 294 ([M+H]$^+$, 100%). The data were in agreement with the literature values.[100]

(*Z*)-2-(Nitrooxy)ethyl 2-(5-fluoro-2-methyl-1-(4-(methylsulfinyl)benzylidene)-1*H*-inden-3-yl)acetate, 140



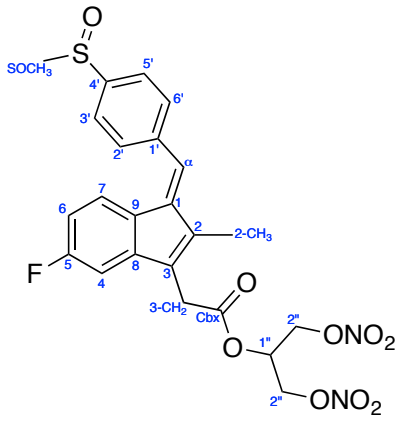
Following general procedure A with sulindac **112** (50 mg, 0.14 mmol) and 2-(nitrooxy)ethanol **136** (17 mg, 0.15 mmol), purification by flash chromatography, eluting with dichloromethane and methanol (99:1), furnished *(Z)-2-(nitrooxy)ethyl 2-(5-fluoro-2-methyl-1-(4-(methylsulfinyl)benzylidene)-1H-inden-3-yl)acetate* **140** (27.1 mg, 0.06 mmol, 44%) as a yellow solid: $\boldsymbol{R_f}$ 0.38 (95:5, CH_2Cl_2:MeOH, UV/cerium phosphomolybdate); **m.p.** 102-104 °C; $\boldsymbol{\nu_{max}}$ (thin film)/cm^{-1} 1635, 1467, 1414, 1372, 1294, 1169; **^{1}H NMR** (300 MHz; $CDCl_3$) δ 7.73 (2H, d, *J* 8.4 Hz, C*H*-3′,5′), 7.67 (2H, d, *J* 8.4 Hz, C*H*-2′,6′), 7.18 (1H, s, C*H*-α), 7.16 (1H, dd, *J* 8.5, 5.1 Hz, C*H*-7), 6.86 (1H, dd, *J* 8.9, 2.4 Hz, C*H*-4), 6.56 (1H, ddd, *J* 8.9, 8.8, 2.4 Hz, C*H*-6), 4.63-4.69 (2H, m, CH_2-2″), 4.37-4.42 (2H, m, CH_2-1″), 3.62 (2H, s, 3-CH_2), 2.80 (3H, s, SOCH_3), and 2.20 (3H, s, 2-CH_3); **^{13}C NMR** (100 MHz; $CDCl_3$) δ 169.9 (quat., carboxyl), 163.5 (quat., d, *J* 246.7 Hz, C-5), 146.5 (quat., d, *J* 8.7 Hz, C-8), 145.7 (quat., C-4′), 141.6 (quat., C-1), 139.7 (quat., C-1′), 138.7 (quat., C-2), 131.2 (quat., C-3), 130.4 (*C*H × 2, C-3′,5′), 129.6 (quat., C-9), 128.6 (*C*H, C-α), 123.9 (*C*H × 2, C-2′,6′), 123.8 (*C*H, d, *J* 8.8 Hz, C-7), 111.0 (*C*H, d, *J* 22.2 Hz, C-6), 106.1 (*C*H, d, *J* 24.0 Hz, C-4), 70.3 (2 × CH_2, C-2″), 61.0 (*C*H, C-1″), 44.1 (CH_3, SO*C*H_3), 31.5 (CH_2, 3-CH_2), 10.7 (CH_3, 2-*C*H_3); **^{19}F {^{1}H} NMR** (376 MHz; $CDCl_3$) δ -113.3 (C*F*); ***m/z*** (ES^+) 468 ($[M+Na]^+$, 100%); **HRMS** *m/z* (ES^+) calcd. for $C_{22}H_{20}FNNaO_6S$ $[M+Na]^+$ requires 468.0893, found 468.0878; **CHN** Anal. calcd. for $C_{22}H_{20}FNO_6S$: C, 59.32; H, 4.53; N, 3.14 Found C, 59.82; H, 4.62; N, 3.22.

(*Z*)-6-(Nitrooxy)hexyl 2-(5-fluoro-2-methyl-1-(4-(methylsulfinyl)benzylidene)-1*H*-inden-3-yl)acetate, 141



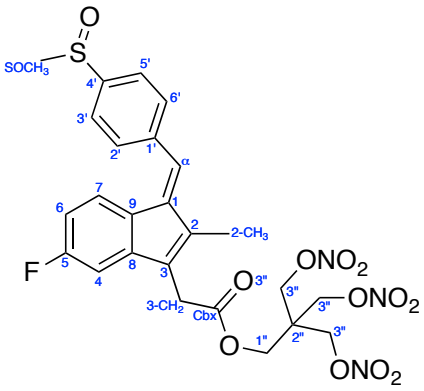
Following general procedure A with sulindac **112** (50 mg, 0.14 mmol) and 6-(nitrooxy)hexan-1-ol **137** (24 mg, 0.15 mmol), purification by flash chromatography, eluting with dichloromethane and methanol (99:1), furnished *(Z)-6-(nitrooxy)hexyl 2-(5-fluoro-2-methyl-1-(4-(methylsulfinyl)benzylidene)-1H-inden-3-yl)acetate* **141** (47 mg, 0.09 mmol, 66%) as a viscous yellow oil: R_f 0.45 (95:5 CH_2Cl_2:MeOH, UV/cerium phosphomolybdate); ν_{max} (thin film)/cm^{-1} 1626, 1467, 1278, 1162; **^{1}H NMR** (300 MHz; $CDCl_3$) δ 7.72 (2H, d, *J* 8.4 Hz, C*H*-3′,5′), 7.66 (2H, d, *J* 8.4 Hz, C*H*-2′,6′), 7.16 (1H, s, C*H*-α), 7.18-7.12 (1H, m, C*H*-7), 6.89 (1H, dd, *J* 8.9, *J* 2.4 Hz, C*H*-4), 6.56 (1H, ddd, *J* 9.0, 8.9, 2.5 Hz, C*H*-6), 4.39 (2H, t, *J* 6.6 Hz, CH_2-6″), 4.11 (2H, t, *J* 6.4 Hz, CH_2-1″), 3.56 (2H, s, 3-CH_2), 2.81 (3H, s, SOCH_3), 2.21 (3H, s, 2-CH_3); 1.56-1.73 (4H, m, 2 × CH_2-2″, 5″) and 1.30-1.43 (4H, m, 2 × CH_2-3″, 4″); **^{13}C NMR** (100 MHz; $CDCl_3$) δ 170.4 (quat., carboxyl), 163.4 (quat., d, *J* 246.5 Hz, C-5), 146.8 (quat., d, *J* 8.7 Hz, C-8), 145.7 (quat., C-4′), 141.7 (quat., C-1), 139.8 (quat., C-1′), 138.3 (quat., C-2), 132.0 (quat., C-3), 130.4 (*C*H × 2, C-3′,5′), 129.6 (quat., C-9), 128.4 (*C*H, C-α), 124.0 (*C*H × 2, C-2′,6′), 123.8 (*C*H, d, *J* 8.8 Hz, C-7), 110.9 (*C*H, d, *J* 22.7 Hz, C-6), 106.3 (*C*H, d, *J* 23.8 Hz, C-4), 73.7 (*C*H_2, C-6″), 64.9 (*C*H_2, C-1″), 44.1 (*C*H_3, SO*C*H_3), 32.1 (*C*H_2, 3-*C*H_2), 28.5 (*C*H_2, C-5″), 26.8 (*C*H_2, C-2″), 25.6 (*C*H_2, C-4″), 25.4 (*C*H_2, C-3″) 10.7 (*C*H_3, 2-*C*H_3); **^{19}F {^{1}H} NMR** (376 MHz; $CDCl_3$) δ -113.5 (C*F*); ***m/z*** (ES^+) 524 ($[M+Na]^+$, 100%); **HRMS** *m/z* (ES^+) calcd. for $C_{26}H_{28}FNNaO_6S$ $[M+Na]^+$ requires 524.1519, found 524.1511; **CHN** Anal. calcd. for $C_{26}H_{28}FNO_6S$: C, 62.26; H, 5.63; N, 2.79. Found C, 62.31; H, 5.71; N, 2.84.

(*Z*)-1,3-*Bis*(nitrooxy)propan-2-yl 2-(5-fluoro-2-methyl-1-(4-(methylsulfinyl)benzylidene)-1*H*-inden-3-yl)acetate, 142



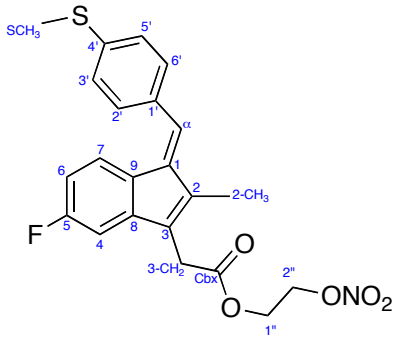
Following general procedure A with sulindac **112** (50 mg, 0.14 mmol) and 1,3-di(nitrooxy)-2-propanol **138** (27 mg, 0.15 mmol), purification by flash chromatography, eluting with dichloromethane and methanol (99:1), furnished *(Z)*-1,3-*bis(nitrooxy)propan-2-yl-2-(5-fluoro-2-methyl-1-(4-(methylsulfinyl)benzylidene)-1H-inden-3-yl)acetate* **142** (58 mg, 0.11 mmol, 80%) as a yellow solid: $\boldsymbol{R_f}$ 0.41 (95:5, CH_2Cl_2:MeOH, UV/cerium phosphomolybdate); **m.p.** 84-86 °C; $\boldsymbol{\nu_{max}}$ (thin film)/cm^{-1} 1743, 1646, 1603, 1467, 1275, 1151; **^{1}H NMR** (300 MHz; $CDCl_3$) δ 7.73 (2H, d, *J* 8.4 Hz, C*H*-3′,5′), 7.67 (2H, d, *J* 8.4 Hz, C*H*-2′,6′), 7.16 (1H, s, C*H*-α), 7.16 (1H, dd, *J* 8.4, 5.1 Hz, C*H*-7), 6.84 (1H, dd, *J* 8.8, 2.4 Hz, C*H*-4), 6.58 (1H, ddd, *J* 8.9, 8.9, 2.4 Hz, C*H*-6), 5.31-5.42 (1H, m, C*H*-1″) 4.75 (2H, dd, *J* 12.6, 4.8 Hz, 2 × CH_AH_B-2), 4.55 (2H, dd, *J* 12.6, 5.8 Hz, 2 × CH_AH_B-2), 3.62 (2H, s, 3-CH_2), 2.80 (3H, s, SOCH_3), and 2.20 (3H, s, 2-CH_3); **^{13}C NMR** (100 MHz; $CDCl_3$) δ 169.3 (quat., carboxyl), 163.5 (quat., d, *J* 246.7 Hz, C-5), 146.3 (quat., d, *J* 8.8 Hz, C-8), 145.8 (quat., C-4′), 141.6 (quat., C-1), 139.7 (quat., C-1′), 139.0 (quat., C-2), 131.6 (quat., C-3), 130.4 (*C*H × 2, C-3′,5′), 129.6 (quat., C-9), 128.9 (*C*H, C-α), 124.0 (*C*H × 2, C-2′,6′), 123.6 (*C*H, d, *J* 8.8 Hz, C-7), 111.2 (*C*H, d, *J* 22.5 Hz, C-6), 106.0 (*C*H, d, *J* 24.4 Hz, C-4), 69.5 (CH_2 × 2, C-2″), 67.3 (*C*H, C-1″), 44.1 (CH_3, SOCH_3), 31.5 (CH_2, 3-CH_2), 10.6 (CH_3, 2-CH_3); **^{19}F {^{1}H} NMR** (376 MHz; $CDCl_3$) δ -113.2 (C*F*); ***m/z*** (ES^+) 543 ($[M+Na]^+$, 100%); **HRMS** *m/z* (ES^+) calcd. for $C_{23}H_{21}FN_2NaO_9S$ $[M+Na]^+$ requires 543.0850, found 543.0840; **CHN** Anal. calcd. for $C_{23}H_{21}FN_2O_9S$: C, 53.08; H, 4.07; N, 5.38. Found C, 53.38; H, 4.18; N, 5.80.

(*Z*)-3-(Nitrooxy)-2,2-*bis*((nitrooxy)methyl)propyl 2-(5-fluoro-2-methyl-1-(4-(methylsulfinyl)benzylidene)-1*H*-inden-3-yl)acetate, 143



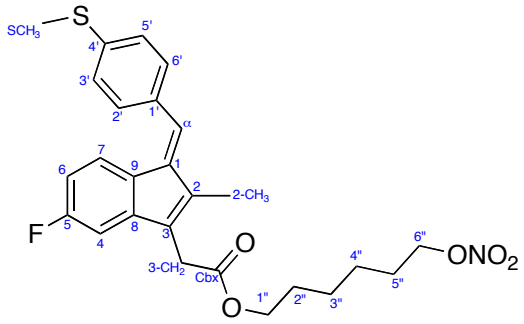
Following general procedure A with sulindac **112** (50 mg, 0.14 mmol) and 3-nitrooxy-2,2-bis(nitrooxymethyl)propan-1-ol **139** (41 mg, 0.15 mmol), purification by flash chromatography, eluting with dichloromethane and methanol (99:1), furnished *(Z)-3-(nitrooxy)*-2,2-*bis((nitrooxy)methyl)propyl 2-(5-fluoro-2-methyl-1-(4-(methylsulfinyl)benzylidene)-1H-inden-3-yl)acetate* **143** (70 mg, 0.12 mmol, 86%) as a yellow gum: $\boldsymbol{R_f}$ 0.39 (95:5, CH_2Cl_2:MeOH); $\boldsymbol{\nu_{max}}$ (thin film)/cm^{-1} 1733, 1652, 1467, 1415, 1273, 1150, 1088; **^{1}H NMR** (300 MHz; $CDCl_3$) δ 7.73 (2H, d, *J* 8.4 Hz, C*H*-3′,5′), 7.67 (2H, d, *J* 8.4 Hz, C*H*-2′,6′), 7.20 (1H, s, C*H*-α), 7.18 (1H, dd, *J* 8.4, 5.1 Hz, C*H*-7), 6.85 (1H, dd, *J* 8.8, 2.4 Hz, C*H*-4), 6.60 (1H, ddd, *J* 8.9, 8.9, 2.4 Hz, C*H*-6), 4.56 (6H, s, 3 × CH_2-3″), 4.21 (2H, s, CH_2-1″), 3.63 (2H, s, 3-CH_2) 2.81 (3H, s, SOCH_3), and 2.21 (3H, s, 2-CH_3); **^{13}C NMR** (100 MHz; $CDCl_3$) δ 169.1 (quat., carboxyl), 163.4 (quat., d, *J* 247.8 Hz, C-5), 146.1 (quat., d, *J* 8.7 Hz, C-8), 145.9 (quat., C-4′), 141.2 (quat., C-1), 139.5 (quat., C-1′), 138.7 (quat., C-2), 130.8 (quat., C-3), 130.4 (*C*H × 2, C-3′,5′), 129.6 (quat., C-9), 129.4 (*C*H, C-α), 124.0 (*C*H × 2, C-2′,6′), 123.9 (*C*H, d, *J* 9.0 Hz, C-7), 111.4 (*C*H, d, *J* 22.5 Hz, C-6), 105.8 (*C*H, d, *J* 24.0 Hz, C-4), 69.2 (3 × *C*H_2, C-3″), 61.9 (*C*H_2, C-1″), 44.1 (*C*H_3, SOCH_3), 42.2 (quat., C-2″), 31.6 (*C*H_2, 3-*C*H_2), 10.6 (*C*H_3, 2-*C*H_3); **^{19}F {^{1}H} NMR** (376 MHz; $CDCl_3$) δ -113.0 (C*F*); ***m/z*** (ES^+) 543 ($[M+Na]^+$, 100%); **HRMS** *m/z* (ES^+) calcd. for $C_{25}H_{24}FN_3NaO_{12}S$ $[M+Na]^+$ requires 632.0962, found 632.0972; **CHN** Anal. calcd. for $C_{25}H_{24}FN_3NaO_{12}S$: C, 49.26; H, 3.97; N, 6.89. Found C, 49.36; H, 4.00; N, 6.91.

(*Z*)-2-(Nitrooxy)ethyl 2-(5-fluoro-2-methyl-1-(4-(methylthio)benzylidene)-1*H*-inden-3-yl)acetate, 144



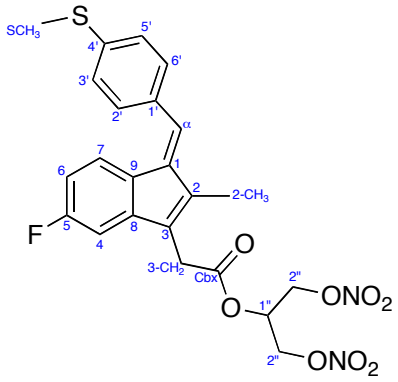
Following general procedure A with sulindac sulfide **115** (50 mg, 0.15 mmol) and 2-(nitrooxy)ethanol **132** (17 mg, 0.15 mmol), purification by flash chromatography, eluting with dichloromethane and methanol (99:1), furnished*(Z)-2-(nitrooxy)ethyl 2-(5-fluoro-2-methyl-1-(4-(methylthio)benzylidene)-1H-inden-3-yl)acetate* **144** (52 mg, 0.12 mmol, 82%) as a yellow solid: R_f 0.86 (95:5, CH_2Cl_2:MeOH, UV/cerium phosphomolybdate); **m.p.** 79-80 °C; ν_{max} (thin film)/cm^{-1} 1741, 1636, 1602, 1567, 1404, 1280, 1151, 1092, 855; **^{1}H NMR** (400 MHz; $CDCl_3$) δ 7.45 (2H, d, *J* 8.4 Hz, C*H*-3′,5′), 7.37 (1H, dd, *J* 8.4, 5.2 Hz, C*H*-7), 7.29 (2H, d, *J* 8.4 Hz, C*H*-2′,6′), 7.15 (1H, s, C*H*-α), 6.86 (1H, dd, *J* 9.0, *J* 2.4 Hz, C*H*-4), 6.59 (1H, ddd, *J* 9.0, 9.0, 2.4 Hz, C*H*-6), 4.67-4.64 (2H, m, CH_2-2″), 4.40-4.37 (2H, m, CH_2-1″), 3.60 (2H, s, 3-CH_2), 2.55 (3H, s, SCH_3), 2.20 (3H, s, 2-CH_3); **^{13}C NMR** (100 MHz; $CDCl_3$) δ 170.0 (quat., carboxyl), 163.1 (quat., d, *J* 242.9 Hz, C-5), 146.2 (quat., d, *J* 8.7 Hz, C-8), 140.0 (quat., C-1), 139.2 (quat., C-2), 138.8 (quat., C-1′), 132.9 (quat., C-3), 130.2 (*C*H, C-α), 130.0 (quat., C-9), 129.9 (*C*H × 2, C-2′,6′), 129.8 (quat., C-4′), 125.9 (*C*H × 2, C-3,5′), 123.7 (*C*H, d, *J* 8.5 Hz, C-7), 110.6 (*C*H, d, *J* 22.4 Hz, C-6), 105.6 (*C*H, d, *J* 23.6 Hz, C-4), 70.2 (CH_2, C-2″), 60.7 (CH_2, C-1″), 31.4 (CH_2, 3-CH_2), 15.4 (CH_3, SCH_3) 10.6 (CH_3, 2-CH_3); **^{19}F {^{1}H} NMR** (376 MHz; $CDCl_3$) δ -114.2 (C*F*); ***m/z*** (ES^+) 452 ($[M+Na]^+$, 100%); **HRMS** *m/z* (ES^+) calcd. for $C_{22}H_{20}FNaO_5S$ $[M+Na]^+$ requires 452.0944, found 452.0940; **CHN** Anal. calcd. for $C_{22}H_{20}FO_5S$: C, 61.53; H, 4.69; N, 3.26. Found C, 61.55; H, 4.73; N, 3.30.

(*Z*)-6-(Nitrooxy)hexyl 2-(5-fluoro-2-methyl-1-(4-(methylthio)benzylidene)-1*H*-inden-3-yl)acetate, 145



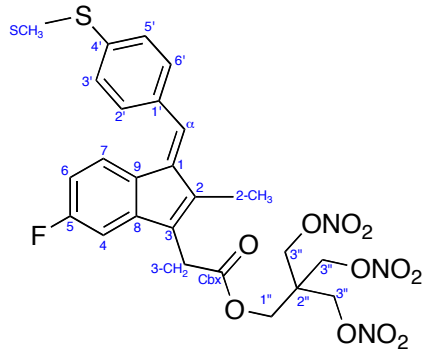
Following general procedure A with sulindac sulfide **115** (50 mg, 0.15 mmol) and 6-(nitrooxy)hexan-1-ol **137** (24 mg, 0.15 mmol), purification by flash chromatography, eluting with dichloromethane and methanol (99:1), furnished *(Z)-6-(nitrooxy)hexyl 2-(5-fluoro-2-methyl-1-(4-(methylthio)benzylidene)-1H-inden-3-yl)acetate* **145** (68 mg, 0.140 mmol, 95%) as a yellow gum: $\boldsymbol{R_f}$ 0.62 (95:5, CH_2Cl_2:MeOH); $\boldsymbol{\nu_{max}}$ (thin film)/cm^{-1} 2940, 1732, 1627, 1466, 1279, 1158, 1092, 1021, 976, 864; **^{1}H NMR** (400 MHz; $CDCl_3$) δ 7.44 (2H, d, *J* 8.2 Hz, C*H*-3′,5′), 7.36 (1H, dd, *J* 8.2, 5.2 Hz, C*H*-7), 7.29 (2H, d, *J* 8.2 Hz, C*H*-2′,6′), 7.14 (1H, s, C*H*-α), 6.89 (1H, dd, *J* 9.0, 2.4 Hz, C*H*-4), 6.58 (1H, ddd, *J* 17.6, 9.0, 2.4 Hz, C*H*-6), 4.38 (2H, t, *J* 6.6 Hz, CH_2-6″), 4.10 (2H, t, *J* 6.5, 3-CH_2), 3.62 (2H, s, 3-CH_2), 2.55 (3H, s, SCH_3), 2.21 (3H, s, 2-CH_3), 1.70-1.58 (4H, CH_2-2″, 5″), 1.40-1.28 (4H, CH_2-3″, 4″); **^{13}C NMR** (100 MHz; $CDCl_3$) δ 170.4 (quat., carboxyl), 163.0 (quat., d, *J* 244.5 Hz, C-5), 146.5 (quat., d, *J* 8.7 Hz, C-8), 140.1 (quat., C-1), 139.2 (quat., C-2), 138.4 (quat., C-1′), 133.0 (quat., C-3), 129.8 (*C*H, C-α), 130.8 (quat., C-9), 129.9 (*C*H × 2, C-2′,6′), 129.0 (quat., C-4′), 126.0 (*C*H × 2, C-3,5′), 123.7 (*C*H, d, *J* 9.0 Hz, C-7), 110.6 (*C*H, d, *J* 23.1 Hz, C-6), 105.8 (*C*H, d, *J* 23.1 Hz, C-4), 73.1 (CH_2, C-6″), 64.8 (CH_2, C-1″), 32.0 (CH_2, 3-CH_2), 28.4 (CH_2, C-5″), 26.7 (CH_2, C-2″), 25.5 (CH_2, C-4″), 25.3 (CH_2, C-3″), 15.4 (CH_3, SCH_3) 10.6 (CH_3, 2-CH_3); **^{19}F {^{1}H} NMR** (376 MHz; $CDCl_3$) δ -114.5 (C*F*); ***m/z*** (ES^+) 508 ($[M+Na]^+$, 100%); **HRMS** *m/z* (ES^+) calcd. for $C_{26}H_{28}FNNaO_5S$ $[M+Na]^+$ requires 508.1570, found 508.1577; **CHN** Anal. calcd. for $C_{26}H_{28}FNO_5S$: C, 64.31; H, 5.81; N, 2.88. Found C, 64.37; H, 5.86; N, 2.91.

(*Z*)-1,3-*Bis*(nitrooxy)propan-2-yl 2-(5-fluoro-2-methyl-1-(4-(methylthio)benzylidene)-1*H*-inden-3-yl)acetate, 146



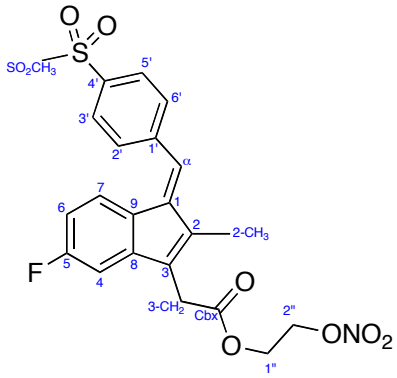
Following general procedure A with sulindac sulfide **115** (50 mg, 0.15 mmol) and 1,3-di(nitrooxy)-2-propanol **138** (27 mg, 0.15 mmol), purification by flash chromatography, eluting with dichloromethane furnished *(Z)*-1,3-*bis*(*nitrooxy)propan-2-yl 2-*(*5-fluoro-2-methyl-1-*(*4-*(methylthio)benzylidene)-1H-inden-3-yl)acetate* **146** (63 mg, 0.125 mmol, 85%) as a yellow solid: $\boldsymbol{R_f}$ 0.67 (95:5, CH_2Cl_2:MeOH, UV/cerium phosphomolybdate); **m.p.** 141-142 °C; $\boldsymbol{\nu_{max}}$ (thin film)/cm^{-1} 2959, 1741, 1638, 1600, 1465, 1312, 1280, 1153, 1091, 854; **^{1}H NMR** (400 MHz; $CDCl_3$) δ 7.45 (2H, d, *J* 8.3 Hz, C*H*-3′,5′), 7.38 (1H, dd, *J* 8.5, 5.2 Hz, C*H*-7), 7.29 (2H, d, *J* 8.3 Hz, C*H*-2′,6′), 7.16 (1H, s, C*H*-α), 6.84 (1H, dd, *J* 9.0, 2.4 Hz, C*H*-4), 6.60 (1H, ddd, *J* 9.0, 9.0, 2.4 Hz, C*H*-6), 5.38 (1H, tt, *J* 5.7, 4.1 Hz, C*H*-1″), 4.74 (2H, dd, *J* 12.6, 4.1 Hz, CH_AH$_B$-2″), 4.55 (2H, dd, *J* 12.7, 5.7 Hz, CH$_A$$H_B$-2″), 3.62 (2H, s, 3-C$H_2$), 2.55 (3H, s, S$CH_3$), 2.20 (3H, s, 2-C$H_3$); **^{13}C NMR** (100 MHz; $CDCl_3$) δ 169.3 (quat., carboxyl), 163.1 (quat., d, *J* 245.6 Hz, C-5), 145.9 (quat., d, *J* 8.7 Hz, C-8), 139.8 (quat., C-1), 139.3 (quat., C-2), 139.1 (quat., C-1′), 132.8 (quat., C-3), 130.5 (*C*H, C-α), 129.3 (quat., C-9), 129.9 (*C*H × 2, C-2′,6′), 129.7 (quat., C-4′), 125.9 (*C*H × 2, C-3,5′), 123.8 (*C*H, d, *J* 8.6 Hz, C-7), 110.7 (*C*H, d, *J* 22.5 Hz, C-6), 105.4 (*C*H, d, *J* 23.6 Hz, C-4), 69.3 (*C*H_2 × 2, C-2″), 67.0 (*C*H_2, C-1″), 31.3 (*C*H_2, 3-*C*H_2), 15.4 (*C*H_3, S*C*H_3) 10.6 (*C*H_3, 2-*C*H_3); **^{19}F {^{1}H} NMR** (376 MHz; $CDCl_3$) δ -114.2 (C*F*); ***m/z*** (ES^+) 527 ([M+Na]$^+$, 100%); **HRMS** *m/z* (ES^+) calcd. for $C_{23}H_{21}FN_2NaO_8S$ [M+Na]$^+$ requires 527.0900, found 527.0911; **CHN** Anal. calcd. for $C_{23}H_{21}FN_2O_7S$: C, 54.76; H, 5.20; N, 5.55. Found C, 54.84; H, 5.33; N, 5.70.

(*Z*)-3-(Nitrooxy)-2,2-*bis*((nitrooxy)methyl)propyl 2-(5-fluoro-2-methyl-1-(4-(methylthio)benzylidene)-1*H*-inden-3-yl)acetate, 147



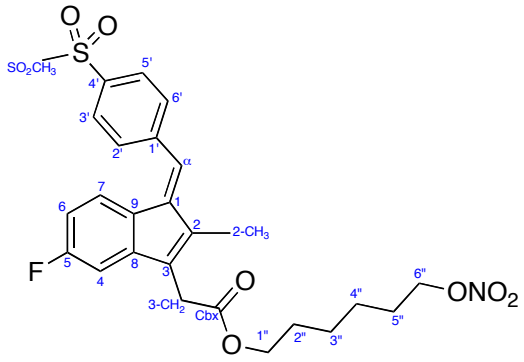
Following general procedure A with sulindac sulfide **115** (50 mg, 0.14 mmol) 3-nitrooxy-2,2-bis(nitrooxymethyl)propan-1-ol **139** (41 mg, 0.15 mmol), purification by flash chromatography, eluting with dichloromethane furnished *(Z)-3-(nitrooxy)*-2,2-*bis*((*nitrooxy)methyl*)propyl *2-(5-fluoro-2-methyl-1-(4-(methylthio)benzylidene)-1H-inden-3-yl)acetate* **137** (80 mg, 0.135 mmol, 92%) as a yellow gum: $\boldsymbol{R_f}$ 0.51 (95:5, CH_2Cl_2:MeOH, UV/cerium phosphomolybdate); $\boldsymbol{\nu_{max}}$ (thin film)/cm^{-1} 2918, 1744, 1647, 1467, 1381, 1277, 1148, 997, 851, 749; **^{1}H NMR** (400 MHz; $CDCl_3$) δ 7.45 (2H, d, *J* 8.2, C*H*-3′,5′), 7.41 (1H, dd, *J* 8.2, 5.2 Hz, C*H*-7), 7.30 (2H, d, *J* 8.3 Hz, C*H*-2′,6′), 7.18 (1H, s, C*H*-α), 6.84 (1H, dd, *J* 8.9, 2.4 Hz, C*H*-4), 6.62 (1H, ddd, *J* 8.9, 8.9, 2.4 Hz, C*H*-6), 4.34 (6H, s, 3 × CH_2-3″), 4.20 (2H, s, CH_2-1″), 3.62 (2H, s, 3-CH_2), 2.55 (3H, s, SCH_3), 2.21 (3H, s, 2-CH_3); **^{13}C NMR** (100 MHz; $CDCl_3$) δ 169.1 (quat., carboxyl), 163.1 (quat., d, *J* 246.9 Hz, C-5), 145.8 (quat., d, *J* 8.3 Hz, C-8), 139.9 (quat., C-1), 139.5 (quat., C-2), 138.8 (quat., C-1′), 132.6 (quat., C-3), 131.0 (*C*H, C-α), 130.9 (quat., C-9), 129.9 (*C*H × 2, C-2′,6′), 129.6 (quat., C-4′), 126.0 (*C*H × 2, C-3,5′), 124.1 (*C*H, d, *J* 8.9 Hz, C-7), 111.0 (*C*H, d, *J* 22.6 Hz, C-6), 105.3 (*C*H, d, *J* 23.8 Hz, C-4), 69.0 (CH_2 × 3, C-3″), 61.7 (CH_2, C-1″), 42.1 (quat., C-2″), 31.5 (CH_2, 3-CH_2), 15.4 (CH_3, SCH_3) 10.5 (CH_3, 2-CH_3); **^{19}F {^{1}H} NMR** (376 MHz; $CDCl_3$) δ -113.9 (C*F*); ***m/z*** (ES^+) 616 ($[M+Na]^+$, 100%); **HRMS** *m/z* (ES^+) calcd. for $C_{25}H_{24}FN_3NaO_{11}S$ $[M+Na]^+$ requires 616.1013, found 616.1020; **CHN** Anal. calcd. for $C_{25}H_{24}FN_3O_{11}S$: C, 50.59; H, 4.08; N, 7.08. Found C, 50.66; H, 4.10; N, 7.13.

(*Z*)-2-(Nitrooxy)ethyl 2-(5-fluoro-2-methyl-1-(4-(methylsulfonyl)benzylidene)-1*H*-inden-3-yl)acetate, 148



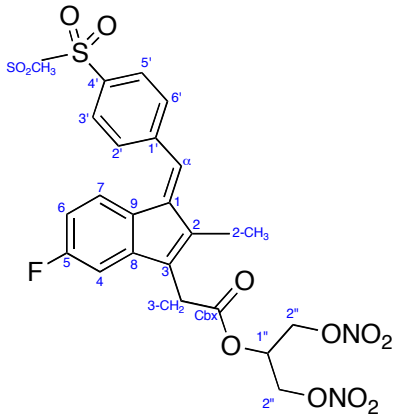
Following general procedure A with sulindac sulfone **116** (50 mg, 0.13 mmol) and 2-(nitrooxy)ethanol **136** (16 mg, 0.14 mmol), purification by flash chromatography, eluting with dichloromethane and methanol (99:1), furnished*(Z)-2-(nitrooxy)ethyl 2-(5-fluoro-2-methyl-1-(4-(methylsulfonyl)benzylidene)-1H-inden-3-yl)acetate* **148** (56 mg, 0.12 mmol, 90%) as a yellow solid: $\boldsymbol{R_f}$ 0.67 (95:5, CH_2Cl_2:MeOH, UV/cerium phosphomolybdate); **m.p.** 141-142 °C; $\boldsymbol{\nu_{max}}$ (thin film)/cm^{-1} 1740, 1635, 1591, 1370, 1310, 1281, 1149, 956, 855, 796; **^{1}H NMR** (300 MHz; $CDCl_3$) δ 8.01 (2H, d, *J* 8.5 Hz, C*H*-3′,5′), 7.70 (2H, d, *J* 8.5 Hz, C*H*-2′,6′), 7.14 (1H, s, C*H*-α), 7.10 (1H, dd, *J* 8.4, 5.2 Hz, C*H*-7), 6.86 (1H, dd, *J* 8.8, 2.3 Hz, C*H*-4), 6.58 (1H, ddd, *J* 8.9, 8.8, 2.3 Hz, C*H*-6), 4.68-4.65 (2H, m, CH_2-2″), 4.42-4.39 (2H, m, CH_2-1″), 3.61 (2H, s, 3-CH_2), 3.14 (3H, s, SO_2CH_3), 2.20 (3H, s, 2-CH_3); **^{13}C NMR** (100 MHz; $CDCl_3$) δ 169.7 (quat., carboxyl), 163.4 (quat., d, *J* 246.9 Hz, C-5), 146.5 (quat., d, *J* 9.3 Hz, C-8), 142.5 (quat., C-4′), 142.3 (quat., C-1′), 139.9 (quat., C-1), 138.5 (quat., C-2), 131.6 (quat., C-3), 130.2 (*C*H × 2, C-2′,6′), 129.4 (quat., C-9), 127.6 (*C*H × 2, C-3,5′), 127.5 (*C*H, C-α), 123.8 (*C*H, d, *J* 9.3 Hz, C-7), 110.1 (*C*H, d, *J* 22.9 Hz, C-6), 106.2 (*C*H, d, *J* 24.1 Hz, C-4), 70.1 (*C*H_2, C-2″), 60.9 (*C*H_2, C-1″), 44.5 (*C*H_3, SO_2CH_3), 31.4 (*C*H_2, 3-*C*H_2), 10.5 (*C*H_3, 2-*C*H_3); **^{19}F {^{1}H} NMR** (376 MHz; $CDCl_3$) δ -112.5 (C*F*); ***m/z*** (ES^+) 484 ($[M+Na]^+$, 100%); **HRMS** *m/z* (ES^+) calcd. for $C_{22}H_{20}FNNaO_7S$ $[M+Na]^+$ requires 484.0842, found 484.0840; **CHN** Anal. calcd. for $C_{22}H_{20}FNO_7S$: C, 57.26; H, 4.37; N, 3.04. Found C, 57.42; H, 4.44; N, 3.11.

(*Z*)-6-(Nitrooxy)hexyl 2-(5-fluoro-2-methyl-1-(4-(methylsulfonyl)benzylidene)-1*H*-inden-3-yl)acetate, 149



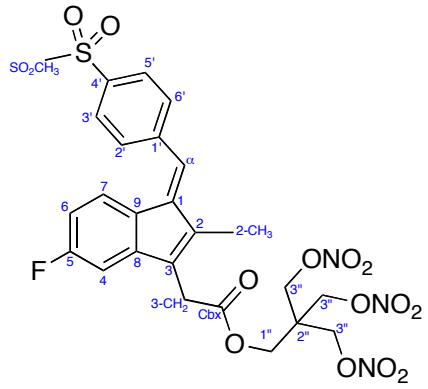
Following general procedure A with sulindac sulfone **116** (50 mg, 0.14 mmol) and 6-(nitrooxy)hexan-1-ol **137** (24 mg, 0.15 mmol), purification by flash chromatography, eluting with dichloromethane and methanol (99:1), furnished *(Z)-6-(nitrooxy)hexyl 2-(5-fluoro-2-methyl-1-(4-(methylsulfonyl)benzylidene)-1H-inden-3-yl)acetate* **149** (63 mg, 0.122 mmol, 90%) as a yellow gum: R_f 0.52 (95:5, CH_2Cl_2:MeOH, UV/cerium phosphomolybdate); ν_{max} (thin film)/cm^{-1} 2866, 2087, 1722, 1628, 1466, 1310, 1280, 1150, 1087, 1021, 960, 864; **^{1}H NMR** (300 MHz; $CDCl_3$) δ 8.00 (2H, d, *J* 8.2 Hz, C*H*-3′,5′), 7.69 (2H, d, *J* 8.2 Hz, C*H*-2′,6′), 7.13 (1H, s, C*H*-α), 7.10 (1H, dd, *J* 8.4, 5.1 Hz, C*H*-7), 6.88 (1H, dd, *J* 8.7, 2.3 Hz, C*H*-4), 6.56 (1H, ddd, *J* 8.9, 8.7, 2.3 Hz, C*H*-6), 4.39 (2H, t, *J* 6.6 Hz, CH_2-6″), 4.12 (2H, t, *J* 6.4, 3-CH_2), 3.56 (2H, s, 3-CH_2), 3.13 (3H, s, SO_2CH_3), 2.20 (3H, s, 2-CH_3), 1.68-1.59 (4H, CH_2-2″, 5″), 1.46-1.39 (4H, CH_2-3″, 6″); **^{13}C NMR** (100 MHz; $CDCl_3$) δ 170.2 (quat., carboxyl), 163.5 (quat., d, *J* 248.0 Hz, C-5), 146.8 (quat., d, *J* 8.7 Hz, C-8), 142.5 (quat., C-4′), 142.3 (quat., C-1′), 139.8 (quat., C-1), 138.1 (quat., C-2), 132.4 (quat., d, *J* 1.7 Hz, C-3), 130.2 (*C*H × 2, C-2′,6′), 129.3 (quat., d, *J* 2.8 Hz, C-9), 127.6 (*C*H × 2, C-3,5′), 127.2 (*C*H, C-α), 123.7 (*C*H, d, *J* 9.1 Hz, C-7), 110.9 (*C*H, d, *J* 22.6 Hz, C-6), 106.4 (*C*H, d, *J* 23.8 Hz, C-4), 73.0 (*C*H_2, C-6″), 64.9 (*C*H_2, C-1), 44.5 (*C*H_3, SO_2CH_3), 31.9 (*C*H_2, 3-*C*H_2), 28.3 (*C*H_2, C-5″), 26.7 (*C*H_2, C-2″), 23.5 (*C*H_2, C-4″), 25.2 (*C*H_2, C-3″), 10.5 (*C*H_3, 2-*C*H_3); **^{19}F {^{1}H} NMR** (376 MHz; $CDCl_3$) δ -112.5 (C*F*); ***m/z*** (ES^+) 540 ($[M+Na]^+$, 100%); **HRMS** *m/z* (ES^+) calcd. for $C_{26}H_{28}FNNaO_7S$ $[M+Na]^+$ requires 540.1468, found 540.1470. **CHN** Anal. calcd. for $C_{26}H_{28}FNNaO_7S$: C, 60.34; H, 5.45; N, 2.71. Found C, 60.36; H, 5.49; N, 2.77.

(*Z*)-1,3-*Bis*(nitrooxy)propan-2-yl 2-(5-fluoro-2-methyl-1-(4-(methylsulfonyl)-benzylidene)-1*H*-inden-3-yl)acetate, 150



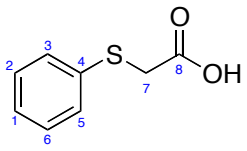
Following general procedure A with sulindac sulfone **116** (50 mg, 0.14 mmol) and 1,3-di(nitrooxy)-2-propanol **138** (27 mg, 0.15 mmol), purification by flash chromatography, eluting with dichloromethane furnished (*Z*)-1,3-*bis*(*nitrooxy)propan-2-yl 2-*(*5-fluoro-2-methyl-1-*(*4-*(methylsulfonyl)benzylidene)-1H-inden-3-yl)acetate* **150** (66 mg, 0.123 mmol, 92%) as a yellow solid: R_f 0.57 (95:5, CH_2Cl_2:MeOH, UV/cerium phosphomolybdate); **m.p.** 92-94 °C; ν_{max} (thin film)/cm^{-1} 2095, 1744, 1640, 1463, 1410, 1277, 1145, 1084, 1014, 963, 833; **^{1}H NMR** (300 MHz; $CDCl_3$) δ 8.00 (2H, d, *J* 8.4 Hz, C*H*-3′,5′), 7.71 (2H, d, *J* 8.4 Hz, C*H*-2′,6′), 7.15 (1H, s, C*H*-α), 7.10 (1H, dd, *J* 8.4, 5.0 Hz, C*H*-7), 6.84 (1H, dd, *J* 8.7, 2.3 Hz, C*H*-4), 6.58 (1H, ddd, *J* 8.9, 8.7, 2.3 Hz, C*H*-6), 5.39 (1H, tt, *J* 5.8, 3.9 Hz, C*H*-1″), 4.76 (2H, dd, *J* 12.7, 3.9, Hz, CH_AH_B-2″), 4.56 (2H, dd, *J* 12.6, 5.8 Hz, CH_AH_B-2″), 3.62 (2H, s, 3-CH_2), 3.14 (3H, s, SO_2CH_3), 2.20 (3H, s, 2-CH_3); **^{13}C NMR** (100 MHz; $CDCl_3$) δ 169.1 (quat., carboxyl), 163.4 (quat., d, *J* 247.1 Hz, C-5), 146.3 (quat., d, *J* 8.9 Hz, C-8), 142.4 (quat., C-4′), 142.1 (quat., C-1′), 139.9 (quat., C-1), 138.8 (quat., C-2), 131.0 (quat., d, *J* 1.8 Hz, C-3), 130.2 (*C*H × 2, C-2′,6′), 129.1 (quat., *J* 2.6 Hz, C-9), 127.7 (*C*H, C-α), 127.6 (*C*H × 2, C-3,5′), 123.8 (*C*H, d, *J* 9.1 Hz, C-7), 111.1 (*C*H, d, *J* 22.4 Hz, C-6), 106.0 (*C*H, d, *J* 24.1 Hz, C-4), 69.3 (CH_2 × 2, C-2″), 67.2 (CH_2, C-1″), 44.5 (CH_3, SO_2CH_3), 31.3 (CH_2, 3-CH_2), 10.5 (CH_3, 2-CH_3); **^{19}F {^{1}H} NMR** (376 MHz; $CDCl_3$) δ -112.7 (C*F*); ***m/z*** (ES^+) 559 ($[M+Na]^+$, 100%); **HRMS** *m/z* (ES^+) calcd. for $C_{23}H_{21}FN_2NaO_{10}S$ $[M+Na]^+$ requires 559.0799, found 559.0803; **CHN** Anal. calcd. for $C_{23}H_{21}FN_2O_{10}S$: C, 51.49; H, 3.95; N, 5.22. Found C, 51.55; H, 4.01; N, 5.28.

(*Z*)-3-(Nitrooxy)-2,2-*bis*((nitrooxy)methyl)propyl 2-(5-fluoro-2-methyl-1-(4-(methylsulfonyl)benzylidene)-1*H*-inden-3-yl)acetate, 151



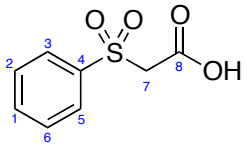
Following general procedure A with sulindac sulfone **116** (50 mg, 0.14 mmol) and 3-nitrooxy-2,2-bis(nitrooxymethyl)propan-1-ol **139** (41 mg, 0.15 mmol), purification by flash chromatography, eluting with dichloromethane, furnished *(Z)-3-(Nitrooxy)-2,2-bis((nitrooxy)methyl)propyl 2-(5-fluoro-2-methyl-1-(4-(methylsulfonyl)benzylidene)-1H-inden-3-yl)acetate* **151** (74 mg, 0.118 mmol, 88%) as a yellow gum; $\boldsymbol{R_f}$ 0.59 (95:5, CH_2Cl_2:MeOH, UV/cerium phosphomolybdate); $\boldsymbol{\nu_{max}}$ (thin film)/cm^{-1} 2912, 2551, 1744, 1646, 1468, 1398, 1279, 1149, 1088, 955, 854, 760; **^{1}H NMR** (300 MHz; $CDCl_3$) δ 8.02 (2H, d, *J* 8.3 Hz, C*H*-3′,5′), 7.70 (2H, d, *J* 8.3 Hz, C*H*-2′,6′), 7.17 (1H, s, C*H*-α), 7.13 (1H, dd, *J* 8.4, 5.1 Hz, C*H*-7), 6.85 (1H, dd, *J* 8.7, 2.2 Hz, C*H*-4), 6.60 (1H, ddd, *J* 8.9, 8.7, 2.2 Hz, C*H*-6), 4.37 (6H, s, 3 × CH_2-3″), 4.21 (2H, s, CH_2-1″), 3.63 (2H, s, 3-CH_2), 3.14 (3H, s, SO_2CH_3), 2.20 (3H, s, 2-CH_3); **^{13}C NMR** (100 MHz; $CDCl_3$) δ 168.9 (quat., carboxyl), 163.4 (quat., d, *J* 247.1 Hz, C-5), 146.3 (quat., d, *J* 8.9 Hz, C-8), 142.2 (quat., C-4′), 141.8 (quat., C-1′), 140.0 (quat., C-1), 138.5 (quat., C-2), 131.2 (quat., d, C-3), 130.2 (*C*H × 2, C-2′,6′), 129.2 (quat., C-9), 128.1 (*C*H, C-α), 127.9 (*C*H × 2, C-3,5′), 123.5 (*C*H, d, *J* 9.2 Hz, C-7), 111.4 (*C*H, d, *J* 22.4 Hz, C-6), 105.8 (*C*H, d, *J* 23.8 Hz, C-4), 69.0 (*C*H_2 × 3, C-3″), 61.9 (*C*H_2, C-1″), 44.5 (*C*H_3, SO_2CH_3), 42.0 (quat., C-2″), 31.5 (*C*H_2, 3-*C*H_2), 10.5 (*C*H_3, 2-*C*H_3); **^{19}F {^{1}H} NMR** (376 MHz; $CDCl_3$) δ -112.0 (C*F*); ***m/z*** (ES^+) 648 ($[M+Na]^+$, 100%); **HRMS** *m/z* (ES^+) calcd. for $C_{25}H_{24}FN_3NaO_{13}S$ $[M+Na]^+$ requires 648.0912, found 648.0916; **CHN** Anal. calcd. for $C_{25}H_{24}FN_3O_{13}S$: C, 48.00; H, 3.87; N, 6.72. Found C, 48.12; H, 3.89; N, 6.75

2-(Phenylthio)acetic acid, 153[152]



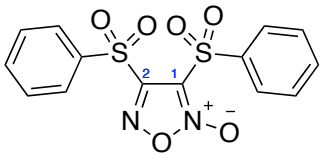
Thiophenol **152** (2.4 g, 2.3 mL, 22.0 mol) and sodium hydroxide (880 mg, 22.0 mol) in 95% aq. EtOH (11 mL) were added dropwise to a solution of chloroacetic acid (2.27 g, 24.0 mol) and sodium carbonate (1.27 g, 6.00 mmol) in distilled water (5 mL) and stirred for 18 h at room temperature. The solution was acidified to pH 2 with aq. HCl solution (3 N) and extracted with ethyl acetate (50 mL). The solvent was removed under reduced pressure to yield 2-(phenylthio)acetic acid **153** (3.57 g, 97%, 21.2 mmol) as white crystals, which was used without any further purification: **m.p.** 60-63 °C; [Lit.[152] 60-62 °C]; **^{1}H NMR** (300 MHz; $CDCl_3$) δ 7.42-7.39 (2H, m, C*H*-3,5), 7.33-7.20 (3H, m, C*H*-1,2,6) and 3.66 (2H, s, CH_2-7); **^{13}C NMR** (100 MHz; $CDCl_3$) δ 175.1 (quat., C-8), 134.5 (quat., C-4), 127.3 (*C*H, C-1), 130.1 (*C*H × 2, C-2,6), 129.8 (*C*H × 2, C-3,5) and 36.6 (CH_2, C-7); ***m/z*** (ES^-) 167 ($[M-H]^-$, 100%). The data were in agreement with the literature values.[399]

2-(Phenylsulfonyl)acetic acid, 154[152]



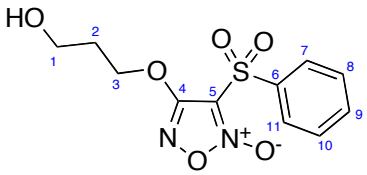
Oxone® (7.37 g, 12.0 mmol) in distilled water (10 mL) was added dropwise to a solution of 2-(phenylthio)acetic acid **153** (1.00 g, 6.0 mmol) in methanol (40 mL) and the resultant suspension stirred for 3 h at room temperature. The volatile components were removed under reduced pressure and the residue was partitioned between ethyl acetate and water (1:1, 100 mL). The organic layer was separated and the solvent removed under reduced pressure to yield 2-(phenylsulfonyl)acetic acid **154** (1.15 g, 5.75 mmol, 96%) as white crystals, which was used without any further purification: **m.p.** 110-113 °C, [Lit.[152] 112-113 °C]; **^{1}H NMR** (300 MHz; $CDCl_3$) δ 7.98 (2H, dt, *J* 7.1, 2.5 Hz, C*H*-3,5), 7.70 (1H, tt, *J* 7.4, 2.5 Hz, C*H*-1*)*, 7.60 (2H, tt, *J* 7.4, 7.1 Hz, C*H*-2, 6) and 4.16 (2H, s, CH_2-7); **^{13}C NMR** (100 MHz; $CDCl_3$) δ 165.8 (quat., C-8), 138.8 (quat., C-4), 135.0 (*C*H, C-1), 129.8 (*C*H × 2, C-2,6), 128.9 (*C*H × 2, C-3,5) and 60.9 (CH_2, C-7); ***m/z*** (ES^-) 199 ($[M-H]^-$, 100%). The data were in agreement with the literature values.[399]

3,4-*Bis*(phenylsulfonyl)-1,2,5-oxadiazole 2-oxide, 53[152]



Fuming nitric acid (27.0 g, 18 mL, 430 mmol) was added dropwise to a solution of 2-(phenylsulfonyl)acetic acid **154** (12.0 g, 60.0 mmol) in glacial acetic acid (35 mL) at 0 °C with stirring. The resulting solution solution was stirred at 0 °C for 15 min, then heated to reflux for 45 min, during which time the solution became a deep red colour. The solution was cooled to room temperature and poured onto ice, resulting in precipitation of crude product. The solid was collected by filtration and recrystallised from 2-propanol to yield 3,4-*bis*(phenylsulfonyl)-1,2,5-oxadiazole 2-oxide **53** (2.88 g, 7.87 mmol, 26%) as white needles: **m.p.** (2-propanol) 154-155 °C, [Lit., 154-156 °C[152]]; **^{1}H NMR** (500 MHz; $CDCl_3$) δ 8.19-8.16 (4H, m, ArC*H* × 4), 7.83-7.79 (2H, m, ArC*H* × 2) and 7.70-7.64 (4H, m, ArC*H* × 4) **^{13}C NMR** (100 MHz; $CDCl_3$) δ 155.6 (quat., C-2), 137.1 (quat.), 136.2 (quat.), 136.1 (*C*H), 135.9 (*C*H), 130.22 (*C*H × 2), 129.9 (*C*H × 2), 129.2 (*C*H × 2) and 115.2 (quat., C-1); ***m/z*** (ES^+), 389 ($[M+Na]^+$, 100%). The data were in agreement with the literature values.[152]

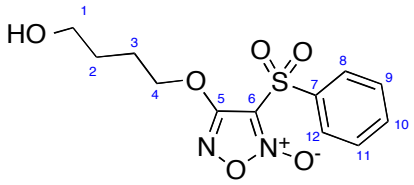
4-(3-Hydroxypropoxy)-3-(phenylsulfonyl)-1,2,5-oxadiazole 2-oxide, 166[152]



1,3-Propanediol **159** (304 mg, 4.0 mmol) and 50% NaOH solution (*w/w*; 350 mg, 4.00 mmol) were added to a stirring solution of 3,4-*bis*(phenylsulfonyl)-1,2,5-oxadiazole 2-oxide **53** (732 mg, 2.00 mmol) in tetrahydrofuran (20 mL) and the resultant solution was stirred for 3 h. The solvent was removed under reduced pressure and the residue was partitioned between distilled water and dichloromethane (1:1, 100 mL). The organic layer was separated and the aqueous layer extracted with dichloromethane (2 × 30 mL). The organic layers were combined and dried over Na_2SO_4, filtered and the solvent removed under reduced pressure to give a brown solid. This solid was purified by silica gel chromatography, eluting with dichloromethane and methanol (100:0 to 99:1), to give 4-(3-hydroxypropoxy)-3-(phenylsulfonyl)-1,2,5-oxadiazole 2-oxide **166** (402 mg, 1.34 mmol, 67%) as a white powder: $\boldsymbol{R_f}$ 0.14 (70:30, PE: EtOAc,

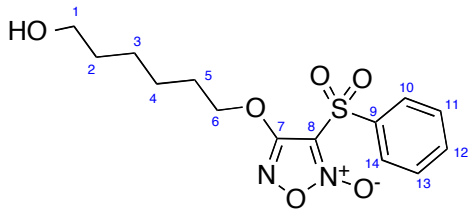
UV/$KMnO_4$); **m.p.** 105-107 °C, [Lit.[152] 105-106 °C]; **^{1}H NMR** (400 MHz; $CDCl_3$) δ 8.06 (2H, d, *J* 8.5 Hz, C*H*-7, 11), 7.77 (1H, tt, *J* 7.3, 1.2 Hz, C*H*-9), 7.63 (2H, t, *J* 7.4 Hz, C*H*-8,10), 4.60 (2H, t, *J* 6.0 Hz, CH_2-3), 3.89 (2H, t, *J* 6.0 Hz, CH_2-1), 2.14 (2H, quintet, *J* 6.0 Hz, CH_2-2) and 1.75 (1H, s, *br*, O*H*), **^{13}C NMR** (100 MHz; $CDCl_3$) δ 158.9 (quat., C-4′), 138.0 (quat., C-6), 135.7 (*C*H, C-9), 129.7 (*C*H × 2, C-7,11), 128.6 (*C*H × 2, C-8,10), 112.2 (*C*H, C-5), 69.3 (*C*H_2, C-3), 59.4 (*C*H_2, C-1) and 31.3 (*C*H_2, C-2); ***m/z*** (ES$^+$) 323 ([M+Na]$^+$, 100%). The data were in agreement with the literature values.[152]

4-(4-Hydroxybutoxy)-3-(phenylsulfonyl)-1,2,5-oxadiazole 2-oxide, 167[152]



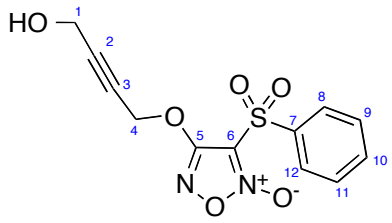
1,4-Butanediol **125** (180 mg, 2.0 mmol) and 50% NaOH solution (*w/w*; 175 mg, 2.00 mmol) were added to a stirring solution of 3,4-*bis*(phenylsulfonyl)-1,2,5-oxadiazole 2-oxide **53** (366 mg, 1.00 mmol) in dry tetrahydrofuran (10 mL) and the resultant solution was stirred for 3 h. The solvent was removed under reduced pressure and the residue was partitioned between distilled water and dichloromethane (1:1, 100 mL). The organic layer was separated and the aqueous layer extracted with dichloromethane (2 × 30 mL). The organic layers were combined and dried over Na_2SO_4, filtered and the solvent removed under reduced pressure to give 4-(4-hydroxybutoxy)-3-(phenylsulfonyl)-1,2,5-oxadiazole 2-oxide **167** (143 mg, 50%) as a white solid which was used without any further purification: ***R$_f$*** 0.21 (70:30, PE: EtOAc, UV/$KMnO_4$); **m.p.** 61-63 °C, [Lit.[152] 61-64 °C]; **^{1}H NMR** (300 MHz; $CDCl_3$) δ 8.08-8.05 (2H, m, C*H*-8,12), 7.77-7.74 (1H, m, C*H*-10), 7.66-7.61 (2H, m, C*H*-9,11), 4.49 (2H, t, *J* 6.3, CH_2-4), 3.81-3.75 (2H, m, CH_2-1), 2.05-1.99 (2H, m, CH_2-3) and 1.81-1.74 (2H, m, CH_2-2); **^{13}C NMR** (100 MHz; $CDCl_3$) δ 159.0 (quat., C-5), 137.9 (quat., C-7), 135.6 (*C*H, C-10), 129.7 (*C*H × 2, C-9,11), 128.6 (*C*H × 2, C-8,12), 111.6 (quat., C-6), 71.5 (*C*H_2, C-4), 62.0 (*C*H_2, C-1), 28.9 and 25.0 (*C*H_2 × 2, C-2,3); ***m/z*** (ES$^+$) 337 ([M+Na]$^+$, 100%). The data were in agreement with the literature values.[152]

4-(6-Hydroxyhexyloxy)-3-(phenylsulfonyl)-1,2,5-oxadiazole 2-oxide, 168



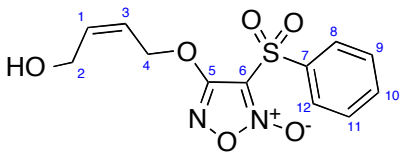
1,6-Hexanediol **160** (475 mg, 4.0 mmol) and 50% NaOH solution (*w/w*; 350 mg, 4.00 mmol) were added to a stirring solution of 3,4-*bis*(phenylsulfonyl)-1,2,5-oxadiazole 2-oxide **53** (732 mg, 2.00 mmol) in tetrahydrofuran (20 mL) and the resultant solution was stirred for 3 h. The solvent was removed under reduced pressure and the residue was partitioned between distilled water and dichloromethane (1:1, 100 mL). The organic layer was separated and the aqueous layer extracted with dichloromethane (2 × 30 mL). The organic layers were combined and dried over Na_2SO_4, filtered and the solvent removed under reduced pressure to give a white solid. This solid was purified by silica gel chromatography, eluting with dichloromethane, to give *4-(6-hydroxyhexyloxy)-3-(phenylsulfonyl)-1,2,5-oxadiazole 2-oxide* **168** (266 mg, 0.78 mmol, 39%) as white crystals: **m.p.** 54-57 °C; $\boldsymbol{R_f}$ 0.33 (70:30, EtOAc:PE, UV/$KMnO_4$); $\boldsymbol{\nu_{max}}$ (thin film)/cm^{-1} 3438, 2921, 1611, 1552, 1491, 1164; **^{1}H NMR** (300 MHz; $CDCl_3$) δ 8.07 (2H, d, *J* 8.5 Hz, C*H*-10,14), 7.77 (1H, tt, *J* 8.5, 1.2 Hz, C*H*-12), 7.63 (2H, t, *J* 7.8 Hz, C*H*-11,13), 4.44 (2H, t, *J* 6.5 Hz, CH_2-6), 3.69 (2H, t, *J* 6.5 Hz, CH_2-1), 1.94-1.87 (2H, m, CH_2-5), 1.66-1.60 (2H, m, CH_2-2), 1.55 (1H, s, *br*, O*H*) and 1.52-1.47 (4H, m, CH_2-3, 4); **^{13}C NMR** (300 MHz; $CDCl_3$) δ 159.0 (quat., C-7), 138.1 (quat., C-9), 135.6 (*C*H, C-12), 129.7 (*C*H × 2, C-11,13), 128.5 (*C*H × 2, C-10,14), 110.5 (quat., C-8), 71.6 (*C*H_2, C-6), 62.7 (*C*H_2, C-1), 32.5 (*C*H_2, C-5), 28.4 (*C*H_2, C-2), 25.5 (*C*H_2, C-4) and 25.3 (*C*H_2, C-3); ***m/z*** (ES^+) 365 ($[M+Na]^+$, 100%); **HRMS** *m/z* (ES^+) calcd. for $C_{14}H_{18}N_2NaO_6S$ $[M+Na]^+$ requires 365.0783, found 365.0790

4-((4-Hydroxybut-2-yn-1-yl)oxy)-3-(phenylsulfonyl)-1,2,5-oxadiazole 2-oxide, 169



But-2-yne-1,4-diol **162** (345 mg, 4.00 mmol) and 50% NaOH solution (*w/w*; 350 mg, 4.00 mmol) were added to a stirring solution of 3,4-*bis*(phenylsulfonyl)-1,2,5-oxadiazole 2-oxide **53** (732 mg, 2.00 mmol) in tetrahydrofuran (20 mL) and the resultant solution was stirred for 3 h. The solvent was removed under reduced pressure and the residue was partitioned between distilled water and dichloromethane (1:1, 100 mL). The organic layer was separated and the aqueous layer extracted with dichloromethane (2 × 30 mL). The organic layers were combined and dried over Na_2SO_4, filtered and the solvent removed under reduced pressure to give a white residue. This residue was purified by silica gel chromatography, eluting with dichloromethane, to give 4-((4-hydroxybut-2-yn-1-yl)oxy)-3-(phenylsulfonyl)-1,2,5-oxadiazole 2-oxide **169** (204 mg, 0.66 mmol, 33%) as a white solid: $\boldsymbol{R_f}$ 0.31 (30:70 EtOAc:PE, UV/$KMnO_4$); **m.p.** 109-112 °C, [Lit.[152] 110-112 °C]; **^{1}H NMR** (300 MHz; $CDCl_3$) δ 8.09 (2H, d, *J* 8.0 Hz, C*H*-8,12), 7.78 (1H, tt, *J* 7.5, 1.8 Hz, C*H*-10), 7.64 (2H, t, *J* 7.5 Hz, C*H*-9,11), 5.12 (2H, t, *J* 1.8 Hz, CH_2-4), 4.36 (2H, t, *J* 1.8 Hz, CH_2-1) and 1.80 (1H, *s, br*, O*H*); **^{13}C NMR** (100 MHz; $CDCl_3$) δ 158.0 (quat., C-5), 137.8 (quat., C-7), 135.8 (*C*H, C-10), 129.7 (*C*H × 2, C-9,11), 128.2 (*C*H × 2, C-8,12), 110.6 (quat., C-6), 88.1 (quat., C-3), 77.6 (quat., C-2), 58.8 (*C*H_2, C-4) and 51.0 (*C*H_2, C-1); ***m/z*** $(ES)^+$ 333 ($[M+Na]^+$, 100%). The data were in agreement with the literature values.[152]

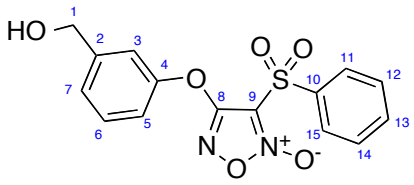
(*Z*)-4-((4-Hydroxybut-2-en-1-yl)oxy)-3-(phenylsulfonyl)-1,2,5-oxadiazole 2-oxide, 170



(*Z*)-But-2-ene-1,4-diol **163** (352 mg, 4.0 mmol) and 50% NaOH solution (*w/w*; 350 mg, 4.00 mmol) were added to a stirring solution of 3,4-*bis*(phenylsulfonyl)-1,2,5-oxadiazole 2-oxide **53** (732 mg, 2.00 mmol) in tetrahydrofuran (20 mL) and the resultant solution was stirred for 3 h. The solvent was removed under reduced pressure and the residue was partitioned between distilled water and dichloromethane (1:1, 100 mL). The organic layer was separated and the aqueous layer extracted with dichloromethane (2 × 30 mL). The organic layers were combined

and dried over Na_2SO_4, filtered and the solvent removed under reduced pressure to give a colourless solid. This solid was purified by silica gel chromatography, eluting with dichloromethane and methanol (100:0 to 99:1) to yield *(Z)-4-((4-hydroxybut-2-en-1-yl)oxy)-3-(phenylsulfonyl)-1,2,5-oxadiazole 2-oxide* **170** (515 mg, 1.64 mmol, 82%) as colourless crystals: R_f 0.21 (30:70, EtOAc:PE, UV/$KMnO_4$); **m.p.** 58-60 °C; ν_{max} (thin film)/cm^{-1} 3413, 1977, 1909, 1615, 1542, 1449, 1257; **^{1}H NMR** (300 MHz; $CDCl_3$) δ 8.06 (2H, d, *J* 8.3 Hz, C*H*-8,12), 7.77 (1H, tt, *J* 8.7, 1.3 Hz, C*H*-10), 7.63 (2H, t, *J* 8.3 Hz, C*H*-9,11), 6.05-5.96 (1H, m, C*H*-3), 5.86-5.78 (1H, m, C*H*-2), 5.08 (2H, d, *J* 6.7 Hz, CH_2-4), 4.33 (2H, t, *J* 5.6 Hz, CH_2-3) and 1.79 (1H, t, *J* 5.7, O*H*) **^{13}C NMR** (300 MHz; $CDCl_3$) δ 158.6 (quat., C-5), 137.9 (quat., C-7), 135.8 (*C*H, C-3), 135.7 (*C*H, C-10), 129.7 (*C*H × 2, C-9,11), 128.6 (*C*H × 2, C-8,12), 123.5 (*C*H, C-2), 110.6 (quat., C-6), 66.7 (*C*H_2, C-4) and 60.4 (*C*H_2, C-1); ***m/z*** (ES^+) 335 ($[M+Na]^+$, 100%); **HRMS** *m/z* (ES^+) calcd. for $C_{12}H_{12}N_2NaO_6S$ $[M+Na]^+$ requires 335.0314, found 335.0322.

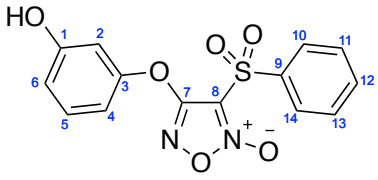
4-(3-(Hydroxymethyl)phenoxy)-3-(phenylsulfonyl)-1,2,5-oxadiazole 2-oxide, 171



3-(Hydroxy)benzyl alcohol **164** (497 mg, 4.00 mmol) and 50% NaOH solution (*w/w*; 350 mg, 4.00 mmol) were added to a stirring solution of 3,4-*bis*(phenylsulfonyl)-1,2,5-oxadiazole 2-oxide **53** (732 mg, 2.00 mmol) in dry tetrahydrofuran (20 mL) and the resultant solution was stirred for 3 h. The solvent was removed under reduced pressure and the residue was partitioned between distilled water and dichloromethane (1:1, 100 mL). The organic layer was separated and the aqueous layer extracted with dichloromethane (2 × 30 mL). The organic layers were combined and dried over Na_2SO_4, filtered and the solvent removed under reduced pressure to give a white residue. This residue was purified by silica gel chromatography, eluting with dichloromethane to yield *4-(3-(hydroxymethyl)phenoxy)*-3-*(phenylsulfonyl)*-1,2,*5-oxadiazole 2-oxide* **171** (376 mg, 1.08 mmol, 54%) as a colourless oil which solidified on standing to a off-white solid: R_f 0.74 (30:70 EtOAc:PE, UV/$KMnO_4$); **m.p.** 75-78 °C; ν_{max} (thin film)/cm^{-1} 3268, 1901, 1609, 1537, 1356, 1644, 1080; **^{1}H NMR** (500 MHz; $CDCl_3$) δ 8.12 (2H, d, *J* 8.0 Hz, C*H*-11,15), 7.81 (1H, tt, *J* 7.5, 1.8 Hz, C*H*), 7.67 (2H, t, *J* 7.5 Hz, C*H*-12,14), 7.45 (1H, t, *J* 7.9 Hz, C*H*-13), 7.36 (1H, s, C*H*), 7.32 (1H, d, *J* 7.9 Hz, C*H*), 7.25 (1H, dd, *J* 7.9, 2.2 Hz, C*H*) and 4.77 (2H, s, CH_2-1); **^{13}C NMR** (100 MHz; $CDCl_3$) δ 158.1 (quat., C-8), 152.9 (quat., C-4), 143.6 (quat., C-2), 138.0 (quat., C-10), 135.8 (*C*H, C-13), 130.1 (*C*H, C-6), 129.8 (*C*H × 2, C-12,14), 128.7 (*C*H × 2, C-11,15), 124.9 (*C*H, C-6), 118.9 (*C*H, C-5), 118.0 (C-7), 110.6 (quat., C-9) and

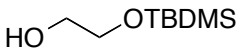
64.4 (*C*H_2, C-1); ***m/z*** (ES^+) 371 ($[M+Na]^+$, 100%); **HRMS** *m/z* (ES^+) calcd. for $C_{15}H_{12}N_2NaO_6S$ $[M+Na]^+$ requires 371.0314 found 371.0320.

4-(3-Hydroxyphenoxy)-3-(phenylsulfonyl)-1,2,5-oxadiazole 2-oxide, 172



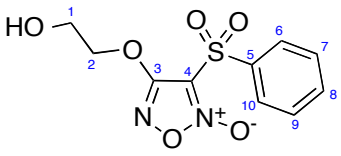
3-Hydroxyphenol **165** (440 mg, 4.00 mmol) and 50% NaOH solution (*w/w*; 350 mg, 4.00 mmol) were added to a stirring solution of 3,4-*bis*(phenylsulfonyl)-1,2,5-oxadiazole 2-oxide **53** (732 mg, 2.00 mmol) in tetrahydrofuran (20 mL) and the resultant solution was stirred for 3 h. The solvent was removed under reduced pressure and the residue was partitioned between distilled water and dichloromethane (1:1, 100 mL). The organic layer was separated and the aqueous layer extracted with dichloromethane (2 × 30 mL). The organic layers were combined and dried over Na_2SO_4, filtered and the solvent removed under reduced pressure to give a deep red oil. Purification by silica gel chromatography, eluting with petroleum ether and ethyl acetate (90:10), yielded colourless glass. This glass was uptaken in chloroform (20 mL) and washed with distilled water (5 × 10 mL) dried over Na_2SO_4, filtered and the solvent evaporated under reduced pressure to yield *4-(3-hydroxyphenoxy)-3-(phenylsulfonyl)-1,2,5-oxadiazole 2-oxide* **172** (218 mg, 0.65 mmol, 33%) as a pale pink glass: $\boldsymbol{R_f}$ 0.40 (30:70 EtOAc:PE, UV/$KMnO_4$); **m.p.** 95-98 °C; $\boldsymbol{\nu_{max}}$ (thin film)/cm^{-1} 3487, 1614, 1533, 1483, 1432, 1356, 1257, 1164, 997, 945; **^{1}H NMR** (400 MHz; $CDCl_3$) δ 8.09 (2H, d, *J* 8.0 Hz, C*H*-10,14), 7.79 (1H, t, *J* 7.3 Hz, C*H*-12), 7.67-7.62 (2H, m, C*H*-11,13), 7.29 (1H, t, *J* 8.2 Hz, C*H*-5), 6.87 (1H, dd, *J* 8.2, 1.8 Hz, C*H*-6), 6.81 (1H, t, *J* 1.8 Hz, C*H*-2) and 6.78 (1H, dd, *J* 8.2, 1.8 Hz, C*H*-4); **^{13}C NMR** (100 MHz; $CDCl_3$) δ 158.2 (quat., C-3), 156.9 (quat., C-7), 153.5 (quat., C-1), 137.9 (quat., C-9), 135.9 (*C*H, C-12), 130.7 (*C*H, C-5), 129.9 (*C*H × 2, C-10,14), 128.6 (*C*H × 2, C-11,13), 113.9 (*C*H, C-6), 111.9 (*C*H, C-4), 110.8 (quat., C-8) and 107.4 (*C*H, C-2); ***m/z*** (ES^+) 357 ($[M+Na]^+$, 100%); **HRMS** *m/z* (ES^+) calcd. for $C_{14}H_{10}N_2NaO_6S$ $[M+Na]^+$ requires 357.0157, found 357.0163

2-((*tert*-Butyldimethylsilyl)oxy)ethanol, 176[324]



TBDMSCl (2.91 g, 19.3 mmol) was added over 1 h to a solution of ethylene glycol **158** (2.33 g, 38.6 mmol), triethylamine (2.5 mL, 24.1 mmol), and DMAP (20 mg, 0.16 mmol) in dichloromethane (150 mL) at 0 °C. The solution was allowed to warm to room temperature and stirred for 16 h. The solution was diluted with dichloromethane (30 mL) and water (30 mL) was added. The biphasic mixture was washed successively with aq. $NaHCO_3$ (saturated, 50 mL), NH_4Cl solution (saturated, 50 mL), water (50 mL) and brine (50 mL), dried over $MgSO_4$, filtered and evaporated under reduced pressure to give a pale yellow oil. This oil was purified by silica gel chromatography, eluting with EtOAc and PE (95:5 PE:EtOAc), to give 2-((*tert*-butyldimethylsilyl)oxy)ethanol **176** (2.15 g, 11.9 mmol, 74%) as a colourless oil: $\boldsymbol{R_f}$ 0.71 (70:30, PE:EtOAc, cerium phosphomolybdate); **^{1}H NMR** (300 MHz; $CDCl_3$) δ 3.64-3.61 (2H, m, CH_2), 3.58-3.54 (2H, m, CH_2), 0.83 (9H, s, CH_3 × 3) and 0.03 (6H, s, CH_3 × 2); **^{13}C NMR** (100 MHz; $CDCl_3$) δ 62.7 (*C*H_2), 61.6 (*C*H_2), 25.8 (*C*H_3 × 3), 18.3 (quat.) and -5.4 (*C*H_3 × 2); ***m/z*** ES^- 175 ($[M-H]^-$, 100%). The data were in agreement with the literature values.[324]

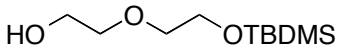
4-(2-Hydroxyethoxy)-3-(phenylsulfonyl)-1,2,5-oxadiazole 2-oxide, 174[152]



2-((*tert*-Butyldimethylsilyl)oxy)ethanol **176** (352 mg, 2.0 mmol) and 50% NaOH solution (*w/w*; 350 mg, 4.00 mmol) were added to a solution of 3,4-*bis*(phenylsulfonyl)-1,2,5-oxadiazole 2-oxide **53** (732 mg, 2.00 mmol) in tetrahydrofuran (20 mL) and the resultant solution was stirred for 3 h. The solvent was removed under reduced pressure and the residue was partitioned between distilled water and dichloromethane (1:1, 100 mL). The organic layer was separated and the aqueous layer extracted with dichloromethane (2 × 30 mL). The organic layers were combined and dried over Na_2SO_4, filtered and the solvent removed under reduced pressure to give a fawn coloured residue (400 mg). This residue was dissolved in methanol (10 mL) and 10-camphorsulfonic acid (400 mg, 1.72 mmol) added. The resulting solution was stirred for 30 min before being quenched with saturated sodium bicarbonate solution (10 mL) and extracted with ethyl acetate (15 mL). The solvent was removed under reduced pressure to give an off

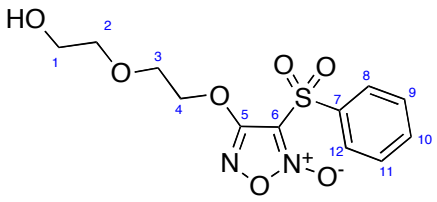
white solid. This solid was purified by silica gel chromatography, eluting with dichloromethane, to give 4-(2-hydroxyethoxy)-3-(phenylsulfonyl)-1,2,5-oxadiazole 2-oxide **174** (340 mg, 0.23 mmol, 60%) as a white solid: R_f 0.17 (70:30, PE: EtOAc, UV/$KMnO_4$); **m.p.** 120-122 °C, [Lit.[152] 118-119 °C]; **^{1}H NMR** (300 MHz; $CDCl_3$) δ 8.08 (2H, d, *J* 8.1 Hz, C*H*-7,9), 7.77 (1H, tt, *J* 6.7, *J* 1.3 Hz, C*H*-8), 7.63 (2H, tt, *J* 8.1, *J* 1.3 Hz, C*H*-6,10), 4.55 (2H, t, *J* 4.5 Hz, CH_2-2), 4.05 (2H, q, *J* 4.5 Hz, CH_2-1) and 2.26 (1H, *s, br*, O*H*); **^{13}C NMR** (100 MHz; $CDCl_3$) δ 159.0 (quat., C-3), 137.9 (quat., C-5), 135.7 (*C*H, C-8), 129.7 (*C*H × 2, C-7, C-9), 128.6 (*C*H × 2, C-6, C-10), 109.9 (quat., C-4), 72.8 (*C*H_2, C-2) and 60.5 (*C*H_2, C-1); ***m/z*** (ES^+) 309 ($[M+Na]^+$, 100%). The data were in agreement with the literature values.[152]

2-(2-((*tert*-butyldimethylsilyl)oxy)ethoxy)ethanol, 177[325]



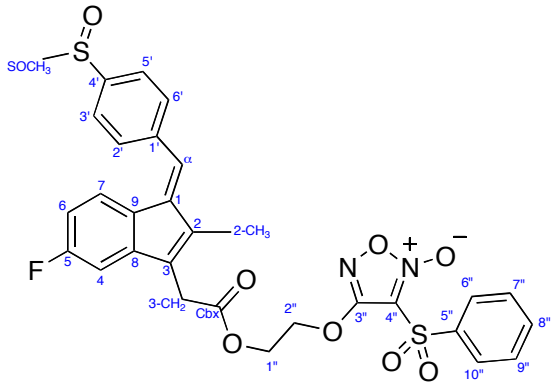
Imidazole (1.26 g, 18.5 mmol) was added to a solution of diethyleneglycol **161** (3.5 mL, 37.0 mmol) in dichloromethane (40 mL) at 0 °C, and the resulting mixture was stirred 5 min. *tert*-Butyldimethylsilyl chloride (2.79 g, 18.5 mmol) was added in one portion and the reaction stirred for 1 h at 0°C and then allowed to warmed to room temperature and stirred overnight. Distilled water (20 mL) was added and the organic layer was washed with aq. HCl solution (1 N, 15 mL), saturated sodium bicarbonate solution (15 mL) and with brine (20 mL). The organic layer was dried over $MgSO_4$, and the solvent removed under reduced pressure to give the crude product. This product was purified by silica gel chromatography, eluting with ethyl acetate and petroleum ether (20:80) give 2-(2-((*tert*-butyldimethylsilyl)oxy)ethoxy)ethanol **177** (2.00 g, 9.08 mmol, 50%) as a colourless oil: R_f 0.25 (70:30, PE: EtOAc, cerium phosphomolybdate); **^{1}H NMR** (300 MHz, $CDCl_3$) δ 3.81-3.77 (2H, m, CH_2-1), 3.74- 3.70 (2H, m, CH_2-4), 3.64-3.60 (4H, m, CH_2-2, 3), 0.91 (9H, s, 3 × CH_3, $SiC(CH_3)_3$) and 0.08 (6H, s, $Si(CH_3)_2$)); **^{13}C NMR** (300 MHz, $CDCl_3$) δ 72.6 (*C*H_2), 72.4 (*C*H_2), 62.9 (*C*H_2), 62.0 (*C*H_2), 25.9 (*C*H_3 × 3), 18.4 (quat.) and -5.3 (*C*H_3 × 2); ***m/z*** $(ES)^-$ 219 ($[M-H]^-$, 100%). The data were in agreement with the literature values.[325]

4-(2-(2-Hydroxyethoxy)ethoxy)-3-(phenylsulfonyl)-1,2,5-oxadiazole 2-oxide, 175[152]



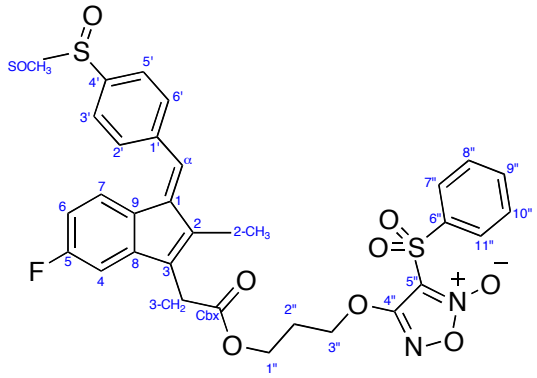
2-(2-*tert*-Butyldimethylsiloxyethoxy)ethanol **177** (400 mg, 2.0 mmol) and 50% NaOH solution (*w/w*; 350 mg, 4.00 mmol) were added to a stirring solution of 3,4-*bis*(phenylsulfonyl)-1,2,5-oxadiazole 2-oxide **53** (732 mg, 2.00 mmol) in tetrahydrofuran (20 mL) and the resultant solution was stirred for 3 h. The solvent was removed under reduced pressure and the residue was partitioned between distilled water and dichloromethane (1:1, 100 mL). The organic layer was separated and the aqueous layer extracted with dichloromethane (2 × 30 mL). The organic layers were combined and dried over Na_2SO_4, filtered and the solvent removed under reduced pressure to give a colourless oil (415 mg). This oil was dissolved in methanol (10 mL) and 10-camphorsulfonic acid (400 mg, 1.72 mmol) was added. The resulting solution was stirred for 30 min before being quenched with saturated sodium bicarbonate solution (10 mL) and extracted with ethyl acetate (20 mL). The solvent was removed under reduced pressure to give an off-white solid. This solid was purified by silica gel chromatography, eluting with dichloromethane and methanol (100:0 to 99:1), to give 4-(2-(2-hydroxyethoxy)ethoxy)-3-(phenylsulfonyl)-1,2,5-oxadiazole 2-oxide **175** (300 mg, 0.91 mmol, 46%) as a white solid: $\boldsymbol{R_f}$ 0.13 (70:30, PE: EtOAc, UV/$KMnO_4$); **m.p.** 58-60 °C, [Lit.[152] 58-60 °C]; **^{1}H NMR** (300 MHz; $CDCl_3$) δ 8.09-8.06 (2H, m, C*H*-8, 12), 7.76 (1H, tt, *J* 6.7, 1.3 Hz, C*H*-10), 7.66-7.60 (2H, m, C*H*-9,11), 4.61-4.58 (2H, m, CH_2-4), 3.96-3.93 (2H, m, CH_2-1), 3.82-3.77 (2H, m, CH_2-3) and 3.73-3.70 (2H, m, CH_2-2); **^{13}C NMR** (100 MHz; $CDCl_3$) δ 159.0 (quat., C-5), 141.1 (quat., C-7), 135.7 (*C*H, C-10), 129.7 (*C*H × 2, C-9,11), 128.6 (*C*H × 2, C-8,12), 109.6 (quat., C-6), 72.7 (*C*H_2, C-4), 70.5 (*C*H_2, C-3), 68.3 (*C*H_2, C-1) and 60.4 (*C*H_2, C-2); ***m/z*** (ES^+) 353 ($[M+Na]^+$, 100%). The data were in agreement with the literature values.[152]

(*Z*)-4-(2-(2-(5-Fluoro-2-methyl-1-(4-(methylsulfinyl)benzylidene)-1*H*-inden-3-yl)acetoxy)ethoxy)-3-(phenylsulfonyl)-1,2,5-oxadiazole 2-oxide, 178



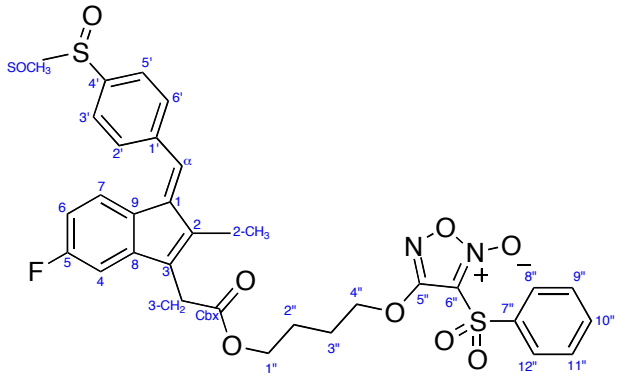
Following general procedure A with sulindac **112** (62 mg, 0.18 mmol) and 4-(2-hydroxyethoxy)-3-(phenylsulfonyl)-1,2,5-oxadiazole 2-oxide **174** (50 mg, 0.18 mmol), purification by flash chromatography, eluting with dichloromethane and methanol (95:5), furnished, *(Z)-4-(2-(2-(5-fluoro-2-methyl-1-(4-(methylsulfinyl)benzylidene)-1H-inden-3-yl)acetoxy)ethoxy)-3-(phenylsulfonyl)*-1,*2,5-oxadiazole 2-oxide* **178** (78 mg, 0.15 mmol, 89%) as a yellow solid: **m.p.** 59-61 °C; $\boldsymbol{R_f}$ 0.47 (95:5, CH_2Cl_2:MeOH, UV/cerium phosphomolybdate); $\boldsymbol{\nu_{max}}$ (thin film)/cm^{-1} 2940, 1735, 1601, 1560, 1459, 1433, 1370, 1241, 1166, 1080; **^{1}H NMR** (400 MHz; $CDCl_3$) δ 8.07 (2H, d, *J* 7.9 Hz, C*H*-6″,10″), 7.79-7.56 (7H, m, C*H*-2′,3′,5′,6′,7″,8″,9″), 7.16 (1H, s, C*H*-α), 7.15 (1H, dd, *J* 8.4, 5.1 Hz, C*H*-7), 6.88 (1H, dd, *J* 8.9, 2.5 Hz, C*H*-4), 6.56 (1H, ddd, *J* 8.9, 8.9, 2.5 Hz, C*H*-6), 4.55 (2H, t, *J* 4.5 Hz, CH_2-1″), 4.04 (2H, t, *J* 4.5 Hz, CH_2-2″), 3.57 (2H, s, 3-CH_2), 2.81 (3H, s, SOCH_3) and 2.21 (3H, s, 2-CH_3); **^{13}C NMR** (100 MHz; $CDCl_3$) δ 170.7 (quat., carboxyl), 163.2 (quat., d, *J* 248.1, C-5), 158.4 (quat., C-3″), 146.7 (quat., d, *J* 8.9, C-8), 145.5 (quat., C-4′), 142.3 (quat., C-1), 139.9 (quat., C-1′), 138.2 (quat., C-2), 137.9 (quat., C-5″), 135.7 (*C*H, C-8″), 131.8 (quat., C-3), 130.3 (*C*H × 2, C-3′,5′), 129.7 (*C*H × 2, C-7″,9″), 129.5 (quat., C-9), 128.2 (*C*H, C-α), 128.6 (*C*H × 2, C-2″,6″), 123.8 (*C*H × 2, C-3′,5′), 123.7 (*C*H, d, *J* 8.9, C-7), 110.8 (*C*H, d, *J* 22.5, C-6), 110.6 (quat., C-4″), 106.2 (*C*H, d, *J* 24.1, C-4), 72.8 (CH_2, C-2″), 60.4 (CH_2, C-1″), 43.9 (CH_3, $SOCH_3$), 31.6 (CH_2, 3-CH_2) and 10.5 (CH_3, 2-CH_3); **^{19}F {^{1}H}** (376 MHz; $CDCl_3$) δ -113.4 (C*F*); ***m/z*** (ES^+) 647 ($[M+Na]^+$, 100%); **HRMS** *m/z* (ES^+) calcd. for $C_{30}H_{25}FN_2NaO_8S_2$ $[M+Na]^+$ requires 647.0934, found 647.0929; **CHN** Anal. calcd. for $C_{30}H_{25}FN_2O_8S_2$: C, 57.68; H, 4.03; N, 4.48. Found 57.85; H, 4.11; N, 4.51.

(*Z*)-4-(3-(2-(5-Fluoro-2-methyl-1-(4-(methylsulfinyl)benzylidene)-1*H*-inden-3-yl)acetoxy)propoxy)-3-(phenylsulfonyl)-1,2,5-oxadiazole 2-oxide, 179



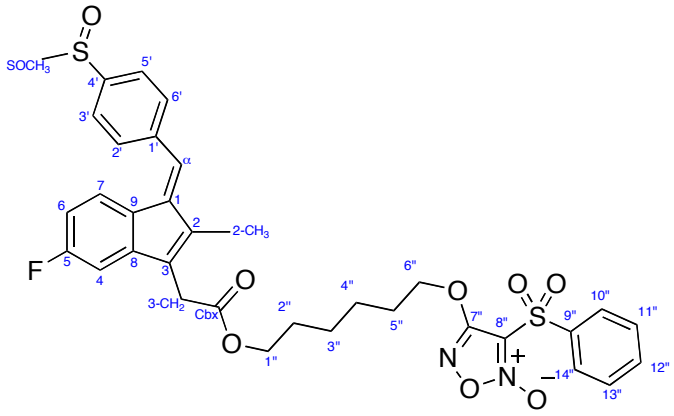
Following general procedure A with sulindac **112** (60 mg, 0.17 mmol) and 4-(3-hydroxypropoxy)-3-(phenylsulfonyl)-1,2,5-oxadiazole 2-oxide **166** (50 mg, 0.17 mmol), purification by flash chromatography, eluting with dichloromethane, furnished *(Z)-4-(3-(2-(5-fluoro-2-methyl-1-(4-(methylsulfinyl)benzylidene)-1H-inden-3-yl)acetoxy)propoxy)-3-(phenylsulfonyl)*-1,*2,5-oxadiazole 2-oxide* **179** (90 mg, 0.14 mmol, 85%) as a yellow solid: **m.p.** 104-106 °C; ***R*$_f$** 0.45 (95:5, CH_2Cl_2:MeOH, UV/cerium phosphomolybdate); **ν_{max}** (thin film)/cm^{-1} 3433, 2935, 1732, 1611, 1552, 1459, 1365, 1255, 1164, 1085; **^{1}H NMR** (500 MHz; $CDCl_3$) δ 8.05 (2H, d, *J* 8.0 Hz, C*H*-7″,11″), 7.80-7.57 (7H, m, C*H*- 2′,3′,4′,5′,8″,9″, 10″), 7.16 (1H, s, C*H*-α), 7.14 (1H, dd, *J* 8.5, 5.1 Hz, C*H*-7), 6.83 (1H, dd, *J* 8.9, 2.4 Hz, C*H*-4), 6.54 (1H, ddd, *J* 8.9, 8.9, 2.4 Hz, C*H*-6), 4.40 (2H, t, *J* 6.0 Hz, CH_2-1″), 4.31 (2H, t, *J* 6.0 Hz, CH_2-3″), 3.59 (2H, s, 3-CH_2), 2.81 (3H, s, SOCH_3) and 2.24-2.10 (5H, m, 2-CH_3 and CH_2-2″); **^{13}C NMR** (100 MHz; $CDCl_3$) δ 170.7 (quat., carboxyl), 163.0 (quat., d, *J* 246.9 Hz, C-5), 158.7 (quat., C-4″), 146.6 (quat., d, *J* 8.5 Hz, C-8), 145.6 (quat., C-4′), 141.5 (quat., C-1), 139.6 (quat., C-1′), 138.3 (quat., C-2), 138.0 (quat., C-6″), 135.7 (*C*H, C-9″), 131.0 (quat., C-3), 130.3 (*C*H × 2, C-2′,6′), 129.2 (*C*H, C-α), 129.7 (*C*H × 2, C-8″,10″), 129.5 (quat., C-9), 128.6 (*C*H × 2, C-7″,11″), 123.9 (*C*H × 2, C-3′,5′), 123.8 (*C*H, d, *J* 8.6, C-7), 110.9 (*C*H, d, *J* 22.4 Hz, C-6), 110.4 (quat., C-5″), 105.9 (*C*H, d, *J* 24.1 Hz, C-4), 67.7 (*C*H_2, C-3″), 60.8 (*C*H_2, C-1″), 43.9 (*C*H_3, SOCH_3), 31.7 (*C*H_2, 3-CH_2), 27.8 (*C*H_2, C-2″) and 10.6 (*C*H_3, 2-CH_3); **^{19}F {^{1}H} NMR** (376 MHz; $CDCl_3$) δ -113.5 (C*F*); ***m/z*** (ES^+) 661 ($[M+Na]^+$, 100%); **HRMS** *m/z* (ES^+) calcd. for $C_{31}H_{27}FN_2NaO_8S_2$ $[M+Na]^+$ requires 661.1091, found 661.1087; **CHN** Anal. calcd. for $C_{31}H_{27}FN_2O_8S_2$: C, 58.30; H, 4.26; N, 4.39. Found C, 58.51; H, 4.32; N, 4.45.

(*Z*)-4-(4-(2-(5-Fluoro-2-methyl-1-(4-(methylsulfinyl)benzylidene)-1*H*-inden-3-yl)acetoxy)butoxy)-3-(phenylsulfonyl)-1,2,5-oxadiazole 2-oxide, 180



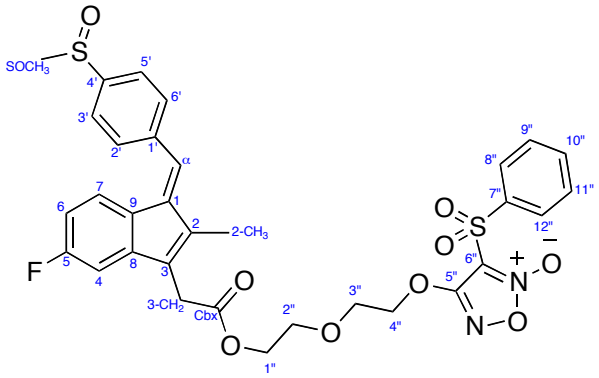
Following general procedure A with sulindac **112** (50 mg, 0.14 mmol) and 4-(4-hydroxybutoxy)-3-(phenylsulfonyl)-1,2,5-oxadiazole 2-oxide **167** (50 mg, 0.15 mmol), purification by flash chromatography, eluting with dichloromethane and methanol (99.5:0.5), furnished *(Z)-4-(4-(2-(5-fluoro-2-methyl-1-(4-(methylsulfinyl)benzylidene)-1H-inden-3-yl)acetoxy)butoxy)-3-(phenylsulfonyl)*-1,2*,5-oxadiazole 2-oxide* **180** (52 mg, 0.08 mmol, 57%) as a yellow solid: **m.p.** 80-82 °C; R_f 0.35 (95:5, CH_2Cl_2:MeOH, UV/cerium phosphomolybdate); ν_{max} (thin film)/cm^{-1} 2930, 1736, 1617, 1550, 1474, 1340, 1249, 1172, 1085; **^{1}H NMR** (300 MHz; $CDCl_3$) δ 8.04 (2H, d, *J* 8.1 Hz, C*H*-8″,12″), 7.70-7.57 (7H, m, C*H*-2′,3′,5′,6′,9″,10′,11″), 7.17 (1H, s, C*H*-α), 7.16 (1H, dd, *J* 8.4, 5.1 Hz, C*H*-7), 6.90 (1H, dd, *J* 8.9, 2.3 Hz, C*H*-4), 6.56 (1H, ddd, *J* 8.9, 8.9, 2.3 Hz, C*H*-6), 4.40 (2H, t, *J* 6.2 Hz, CH_2-1″), 4.20 (2H, t, *J* 5.9 Hz, CH_2-4″), 3.60 (2H, s, 3-CH_2), 2.81 (3H, s, SOCH_3), 2.22 (3H, s, 2-CH_3), 2.05-1.96 (2H, m, CH_2-2″) and 1.87-1.76 (2H, m, CH_2-3); **^{13}C NMR** (100 MHz; $CDCl_3$) δ 170.4 (quat., carboxyl), 163.6 (quat., d, *J* 245.0 Hz, C-5), 159.5 (quat., C-5″), 146.5 (quat., d, *J* 8.9 Hz, C-8), 139.9 (quat., C-1), 141.0 (quat., C-4′), 139.2 (quat. × 2, C-7″, C-1′), 138.4 (quat., C-2), 135.6 (*C*H, C-10″), 132.9 (quat., C-3), 129.9 (*C*H × 2, C-9″,11″), 129.0 (*C*H, C-α), 129.9 (*C*H × 2, C-2′,6′), 129.7 (quat., C-9), 129.9 (*C*H × 2, C-8″,12″), 124.3 (*C*H × 2, C-3′,5′), 123.7 (*C*H, d, *J* 8.9, C-7), 112.8 (quat., C-6″), 111.2 (*C*H, d, *J* 22.3 Hz, C-6), 106.5 (*C*H, d, *J* 23.1 Hz, C-4), 71.3 (*C*H_2, C-4″), 64.6 (*C*H_2, C-1″), 44.3 (*C*H_3, SOCH_3), 32.2 (*C*H_2, 3-CH_2), 25.6 (*C*H_2, C-3″), 25.4 (*C*H_2, C-2″) and 11.0 (*C*H_3, 2-CH_3); **^{19}F {^{1}H} NMR** (376 MHz; $CDCl_3$) δ -113.5, s (C*F*); ***m/z*** (ES$^+$) 675 ([M+Na]$^+$, 100%); **HRMS** *m/z* (ES$^+$) calcd. for $C_{32}H_{29}FN_2O_8NaS_2$ [M+Na]$^+$ requires 675.1247, found 675.1238. **CHN** Anal. calcd. for $C_{32}H_{29}FN_2O_8S_2$: C, 58.88; H, 4.48; N, 4.29. Found C, 58.95; H, 4.51; N, 4.30.

(*Z*)-4-((6-(2-(5-Fluoro-2-methyl-1-(4-(methylsulfinyl)benzylidene)-1*H*-inden-3-yl)acetoxy)hexyl)oxy)-3-(phenylsulfonyl)-1,2,5-oxadiazole 2-oxide, 181



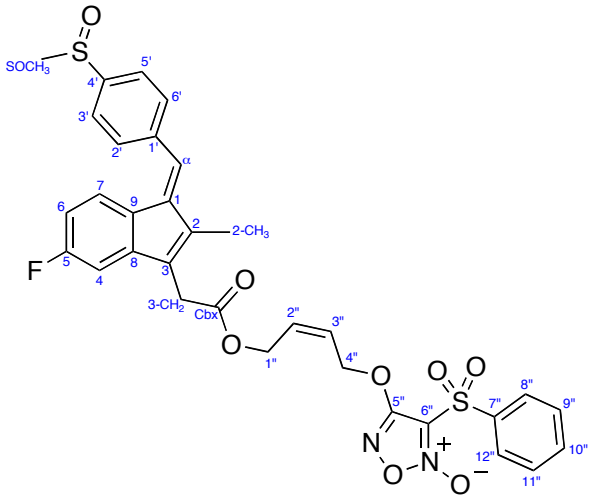
Following general procedure A with sulindac **112** (52 mg, 0.15 mmol) and 4-((6-hydroxyhexyl)oxy)-3-(phenylsulfonyl)-1,2,5-oxadiazole 2-oxide **168** (50 mg, 0.15 mmol), purification by flash chromatography, eluting with dichloromethane and methanol (99:1), furnished (*Z*)-4-((6-(2-(5-*fluoro*-2-*methyl*-1-(4-(*methylsulfinyl*)*benzylidene*)-1*H*-*inden*-3-*yl*)*acetoxy*)*hexyl*)*oxy*)-3-(*phenylsulfonyl*)-1,2,5-*oxadiazole 2-oxide* **181** (83 mg, 0.12 mmol, 83%) as a yellow solid: **m.p.** 42-44 °C; R_f 0.40 (99:5, CH_2Cl_2:MeOH, UV/cerium phosphomolybdate); ν_{max} (thin film)/cm^{-1} 2926, 1734, 1614, 1552, 1464, 1447, 1356, 1260, 1169, 1085; **^{1}H NMR** (400 MHz; $CDCl_3$) δ, 8.05 (2H, d, *J* 8.5, C*H*-10″,14″), 7.76-7.72 (3H, m, C*H*-3′,5′, 12″), 7.67 (2H, d, *J* 8.3 Hz, C*H*-2′,6′) 7.61 (2H, t, *J* 7.9 Hz, C*H*-11″, 13″), 7.17 (1H, s, C*H*-α), 7.16 (1H, dd, *J* 8.3, 5.2 Hz, C*H*-7), 6.90 (1H, dd, *J* 8.9, 2.4 Hz, C*H*-4), 6.56 (1H, ddd, *J* 8.9, 8.9, 2.4 Hz, C*H*-6), 4.39 (2H, t, *J* 6.5 Hz, CH_2-1″), 4.14 (2H, t, *J* 6.5 Hz, CH_2-6″), 3.58 (2H, s, 3-CH_2), 2.82 (3H, s, SOCH_3), 2.22 (3H, s, 2-CH_3), 1.87-1.80 (2H, m, CH_2-2″), 1.71-1.64 (2H, m, CH_2-5″) and 1.50-1.36 (4H, m, CH_2 × 2, CH_2-3″, CH_2-4″); **^{13}C NMR** (100 MHz; $CDCl_3$) δ 170.5 (quat., carboxyl), 163.1 (quat., d, *J* 243.6 Hz, C-5), 159.0 (quat., C-7″), 146.5 (quat., d, *J* 8.4 Hz, C-8), 140.0 (quat., C-1), 139.2 (quat. × 2, C-2, C-4′), 138.4 (quat., C-1′), 138.1 (quat., C-9″), 135.6 (*C*H, C-12″), 132.9 (quat., C-3), 130.8 (*C*H, C-α), 129.9 (*C*H × 2, C-3′,5′), 129.8 (quat., C-9), 129.6 (*C*H × 2, C-10″,14″), 128.5 (*C*H × 2, C-11″,13″), 125.9 (*C*H × 2, C-2′,6′), 123.7 (*C*H, d, *J* 8.8 Hz, C-7), 110.5 (*C*H, d, *J* 22.6 Hz, C-6), 110.5 (quat., C-8″), 105.8 (*C*H, d, *J* 23.7 Hz, C-4), 71.4 (*C*H_2, C-6″), 64.8 (*C*H_2, C-1″), 32.0 (*C*H_2, 3-CH_2), 28.5 (*C*H_2, C-5″), 28.3 (*C*H_2, C-2″), 25.5 (*C*H_2, C-4″), 25.2 (*C*H_2, C-3″), 15.4 (*C*H_3, SCH_3) and 10.6 (*C*H_3, 3-CH_3); **^{19}F {^{1}H} NMR** (376 MHz; $CDCl_3$) δ, -113.5 (C*F*); ***m/z*** (ES^+) 703 ($[M+Na]^+$, 100%); **HRMS** *m/z* (ES^+) calcd. for $C_{34}H_{33}FN_2NaO_8S_2$ $[M+Na]^+$ requires 703.1560, found 703.1555; **CHN** Anal. calcd. for $C_{34}H_{33}FN_2O_8S_2$: C, 59.99; H, 4.89; N, 4.12. Found C, 60.08; H, 5.00; N, 4.18.

(*Z*)-4-(2-(2-(2-(5-Fluoro-2-methyl-1-(4-(methylsulfinyl)benzylidene)-1*H*-inden-3-yl)acetoxy)ethoxy)ethoxy)-3-(phenylsulfonyl)-1,2,5-oxadiazole 2-oxide, 182



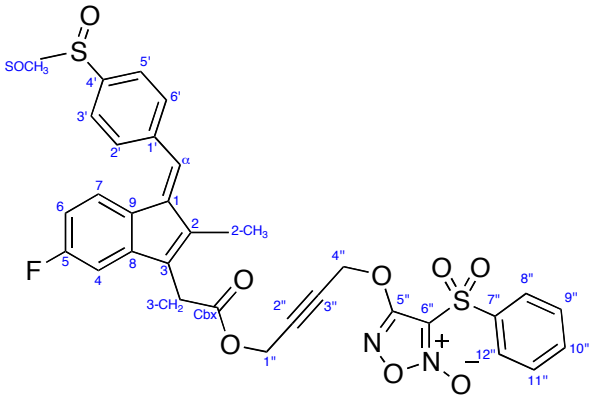
Following general procedure A with sulindac **112** (51 mg, 0.16 mmol) and 4-(2-(3-hydroxyethoxy)ethoxy)-3-(phenylsulfonyl)-1,2,5-oxadiazole 2-oxide **175** (50 mg, 0.15 mmol) purification by flash chromatography, eluting with dichloromethane and methanol (95:5), furnished, *(Z)-4-(2-(2-(2-(5-fluoro-2-methyl-1-(4-(methyl-sulfinyl)benzylidene)-1H-inden-3-yl)acetoxy)-ethoxy)ethoxy)-3-(phenylsulfonyl)*-1,2*,5-oxadiazole 2-oxide* **182** (36 mg, 0.10 mmol, 38%) as a yellow solid: **m.p.** 56-58 °C; ***R*$_f$** 0.37 (95:5, CH_2Cl_2:MeOH, UV/cerium phosphomolybdate); **ν_{max}** (thin film)/cm^{-1} 2935, 1732, 1609, 1550, 1459, 1370, 1164; **^{1}H NMR** (300 MHz; $CDCl_3$) δ 8.07-8.04 (2H, m, C*H*-8″,12″), 7.79-7.56 (7H, m, C*H*-2′,3′,5′,6′, 9″,10″,11″), 7.15 (1H, s, C*H*-α), 7.14 (1H, dd, *J* 8.4, 5.1 Hz, C*H*-7), 6.92 (1H, dd, *J* 9.0, *J* 2.4, C*H*-4), 6.54 (1H, ddd, *J* 9.0, 17.6, *J* 2.4, C*H*-6), 4.52-4.49 (2H, m, CH2-1″), 4.33-4.30 (2H, m, CH_2-4), 3.87-3.84 (2H, m, CH_2-2″), 3.80-3.77 (2H, m, CH_2-3″), 3.62 (2H, s, 3-CH_2), 2.81 (3H, s, SOCH_3) and 2.21 (3H, s, 2-CH_3); **^{13}C NMR** (100 MHz; $CDCl_3$) δ 170.2 (quat., carboxyl), 163.4 (quat., d, *J* 244.5 Hz, C-5), 158.79 (quat., C-5″), 146.7 (quat., d, *J* 8.8, C-8), 145.6 (quat., C-4′), 141.6 (quat., C-1), 139.6 (quat., C-1′), 138.4 (quat., C-2), 138.1 (quat., C-7″), 135.6 (*C*H, C-10″), 131.7 (quat., C-3), 130.3 (*C*H × 2, C-2′,6′), 129.7 (*C*H × 2, C-9″,11″), 129.3 (quat., C-9), 128.6 (*C*H × 2, C-3′,5′), 128.4 (*C*H, C-α), 127.7 (*C*H × 2, C-8″,12″), 123.7 (*C*H, d, *J* 8.8, C-7), 110.8 (*C*H, d, *J* 23.0 Hz, C-6), 110.5 (quat., C-6″), 106.2 (*C*H, d, *J* 23.8 Hz, C-4), 70.5 (*C*H_2, C-4″), 69.4 (*C*H_2, C-1″), 68.3 (*C*H_2, C-2″), 64.0 (*C*H_2, C-3″), 43.9 (*C*H_3, SOCH_3), 31.7 (*C*H_2, 3-CH_2) and 10.6 (*C*H_3, 2-CH_3); **^{19}F {^{1}H} NMR** (376 MHz; $CDCl_3$) δ -113.5 (C*F*); ***m/z*** (ES^+) 691 ($[M+Na]^+$, 100%); **HRMS** *m/z* (ES^+) calcd. for $C_{32}H_{29}FN_2NaO_9S_2$, $[M+Na]^+$ requires 691.1196, found 691.1200; **CHN** Anal. calcd. for $C_{32}H_{29}FN_2O_9S_2$: C, 57.48; H, 4.37; N, 4.19. Found C, 57.64; H, 4.40; N, 4.27.

4-(((*Z*)-4-(2-((*Z*)-5-Fluoro-2-methyl-1-(4-(methylsulfinyl)benzylidene)-1*H*-inden-3-yl)acetoxy)but-2-en-1-yl)oxy)-3-(phenylsulfonyl)-1,2,5-oxadiazole 2-oxide, 183



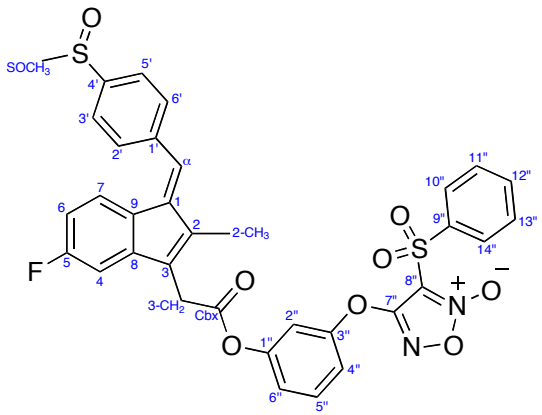
Following general procedure A with sulindac **112** (57 mg, 0.17 mmol) and (*Z*)-4-((4-hydroxybut-2-en-1-yl)oxy)-3-(phenylsulfonyl)-1,2,5-oxadiazole 2-oxide **170** (50 mg, 0.17 mmol), purification by flash chromatography, eluting with dichloromethane and methanol (99:1), furnished *4-(((Z)-4-(2-((Z)-5-fluoro-2-methyl-1-(4-(methylsulfinyl)benzylidene)-1H-inden-3-yl)acetoxy)but-2-en-1-yl)oxy)-3-(phenylsulf-onyl)*-1,2*,5-oxadiazole 2-oxide* **183** (96 mg, 0.15 mmol, 93%) as a yellow solid: **m.p.** 98-100 °C; R_f 0.36 (95:5 CH_2Cl_2:MeOH, UV/cerium phosphomolybdate); ν_{max} (thin film)/cm^{-1} 3059, 2921, 1732, 1609, 1545, 1456, 1407, 1255, 1159, 1083, 1046; **^{1}H NMR** (500 MHz; $CDCl_3$) δ 8.05 (2H, d, *J* 8.2, C*H*-8″,12″), 7.78-7.57 (7H, m C*H*-2′,3′,5′,6′,9″,10″,11″), 7.15 (1H, s, C*H*-α), 7.15 (1H, dd, *J* 8.4, 5.1, C*H*-7), 6.87 (1H, dd, *J* 8.9, 2.4 Hz, C*H*-6), 6.56 (1H, ddd, *J* 8.9, 8.9, 2.5 Hz, C*H*-4), 5.92-5.88 (2H, m, C*H*-2″,3″), 5.01 (2H, d, *J* 5.1 Hz, 3-CH_2), 4.76 (2H, d, *J* 5.2 Hz, CH_2-4), 3.60 (2H, s, 3-CH_2), 2.81 (3H, s, SOCH_3) and 2.21 (3H, s, 2-CH_3); **^{13}C NMR** (100 MHz; $CDCl_3$) δ 169.9 (quat., carboxyl), 163.4 (quat., d, *J* 245.8 Hz, C-5), 158.5 (quat., C-5″), 146.6 (quat., d, *J* 8.8, C-8), 145.6 (quat., C-4′), 141.6 (quat., C-1), 139.6 (quat., C-1′), 138.4 (quat., C-2), 138.0 (quat., C-7″), 135.7 (*C*H, C-10″), 131.5 (quat., C-3), 130.3 (*C*H × 2, C-3′,5′), 130.0 (*C*H, C-3″), 129.5 (quat., C-9), 129.7 (*C*H × 2, C-9″,11″), 128.6 (*C*H × 2, C-8″,12″), 128.5 (*C*H, C-α), 126.2 (*C*H, C-2″), 123.9 (*C*H × 2, C-2′,6′), 123.8 (*C*H, d, *J* 9.8 Hz, C-7), 112.0 (quat., C-6″), 110.9 (*C*H, d, *J* 22.9 Hz, C-6), 106.1 (*C*H, d, *J* 23.8 Hz, C-4), 66.5 (*C*H_2, C-4″), 60.6 (C-1″), 43.9 (*C*H_3, SOCH_3), 31.7 (*C*H_2, 3-CH_2) and 10.6 (*C*H_3, 2-CH_3); **^{19}F {^{1}H} NMR** (376 MHz; $CDCl_3$) δ -112.8 (C*F*); ***m/z*** (ES^+) 673 ($[M+Na]^+$, 100%); **HRMS** *m/z* (ES^+) calcd. for $C_{32}H_{27}FN_2NaO_8S_2$ $[M+Na]^+$ requires 673.1091, found 673.1089; **CHN** Anal. calcd. for $C_{32}H_{27}FN_2O_8S_2$: C, 59.07; H, 4.18; N, 4.31. Found C, 59.20; H, 4.28; N, 4.36.

(*Z*)-4-((4-(2-(5-Fluoro-2-methyl-1-(4-(methylsulfinyl)benzylidene)-1*H*-inden-3-yl)acetoxy)but-2-yn-1-yl)oxy)-3-(phenylsulfonyl)-1,2,5-oxadiazole 2-oxide, 184



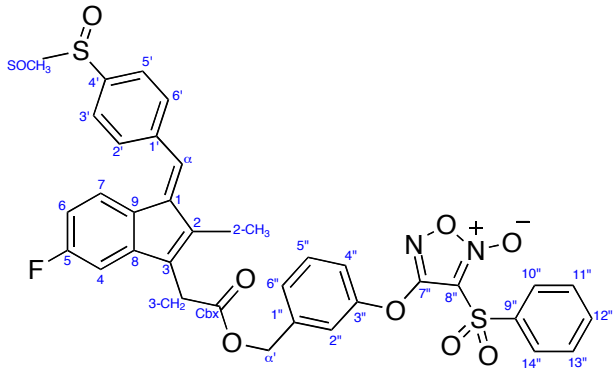
Following general procedure A with sulindac **112** (57 mg, 0.17 mmol) and 4-((4-hydroxybut-2-yn-1-yl)oxy)-3-(phenylsulfonyl)-1,2,5-oxadiazole 2-oxide **169** (50 mg, 0.17 mmol), purification by flash chromatography, eluting with dichloromethane and methanol (99:1), furnished *(Z)-4-((4-(2-(5-fluoro-2-methyl-1-(4-(methylsulfinyl)benzylidene)-1H-inden-3-yl)acetoxy)but-2-yn-1-yl)oxy)-3-(phenylsulfonyl)*-1,2*,5-oxadiazole 2-oxide* **184** (71 mg, 0.11 mmol, 68%) as a yellow solid: **m.p.** 89-90 °C; R_f 0.29 (95:5 CH_2Cl_2:MeOH, UV/cerium phosphomolybdate); ν_{max} (thin film)/cm^{-1} 2926, 1732, 1614, 1552, 1469, 1452, 1359, 1255, 1155, 1080; **^{1}H NMR** (400 MHz; $CDCl_3$) δ, 8.07 (2H, d, *J* 8.3 Hz, C*H*-8″,12″), 7.80-7.60 (7H, m, C*H*-2′,3′,5′,6′,9″, 10″,11″), 7.18 (1H, s, C*H*-α), 7.16 (1H, dd, *J* 5.2, 8.4 Hz, C*H*-7), 6.89 (1H, dd, *J* 8.9, 2.3 Hz, C*H*-6), 6.57 (1H, ddd, *J* 9.0, 8.9, 2.3, C*H*-4), 5.10 (2H, t, *J* 1.7 Hz, CH_2-1″), 4.78 (2H, t, *J* 1.7 Hz, CH_2-4), 3.63 (2H, s, 3-CH_2), 2.82 (3H, s, SOCH_3) and 2.22 (3H, s, 2-CH_3); **^{13}C NMR** (100 MHz; $CDCl_3$) δ, 169.4 (quat., carboxyl), 163.4 (quat., d, *J* 246.5 Hz, C-5), 158.3 (quat., C-5″), 146.6 (quat., d, *J* 7.8 Hz, C-8), 145.6 (quat., C-4′), 141.5 (quat., C-1), 139.9 (quat., C-1′), 138.6 (quat., C-2), 137.8 (quat., C-7″), 135.7 (*C*H, C-10″), 131.1 (quat., C-3), 130.3 (*C*H × 2, C-3′,5′), 129.7 (*C*H × 2, C-8″,12″), 129.5 (quat., C-9), 128.7 (*C*H × 2, C-9″,11″), 128.6 (*C*H, C-α), 123.9 (*C*H × 2, C-2′,6′), 123.7 (*C*H, d, *J* 9.4 Hz, C-7), 110.9 (*C*H, d, *J* 22.7 Hz, C-6), 110.6 (quat., C-6″), 106.1 (*C*H, d, *J* 24.0 Hz, C-4), 83.7 (quat., C-3″), 78.9 (quat., C-2″), 60.4 (*C*H_2, C-4″), 58.6 (*C*H_2, C-1″), 44.0 (*C*H_3, $SOCH_3$), 31.5 (*C*H_2, 3-CH_2) and 10.6 (*C*H_3, 3-CH_3); **^{19}F {^{1}H} NMR** (376 MHz; $CDCl_3$) δ -113.4 (C*F*); ***m/z*** (ES^+) 671 ($[M+Na]^+$, 100%); **HRMS** *m/z* (ES^+) calcd. for $C_{32}H_{25}FN_2NaO_8S_2$ $[M+Na]^+$ requires 671.0934, found 671.0926; **CHN** Anal. calcd. for $C_{32}H_{25}FN_2O_8S_2$: C, 59.25; H, 3.88; N, 4.32. Found C, 59.30; H, 4.00; N, 4.29.

(*Z*)-4-(3-(2-(5-Fluoro-2-methyl-1-(4-(methylsulfinyl)benzylidene)-1*H*-inden-3-yl)acetoxy)phenoxy)-3-(phenylsulfonyl)-1,2,5-oxadiazole 2-oxide, 186



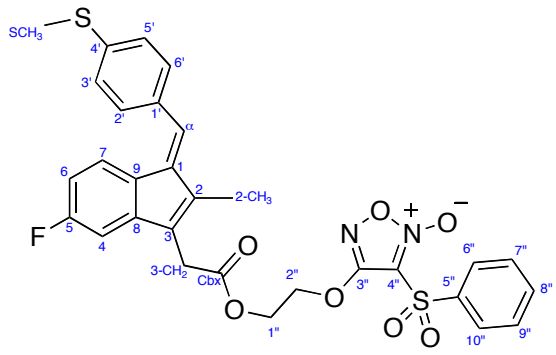
Following general procedure A with sulindac **112** (53 mg, 0.15 mmol) and 4-(3-hydroxyphenoxy)-3-(phenylsulfonyl)-1,2,5-oxadiazole 2-oxide **172** (50 mg, 0.15 mmol), purification by flash chromatography, eluting with dichloromethane and methanol (99.5:0.5), furnished *(Z)-4-(3-(2-(5-fluoro-2-methyl-1-(4-(methylsulfinyl)benzylidene) -1H-inden-3-yl)acetoxy)phenoxy)-3-(phenylsulfonyl)*-1,2,*5-oxadiazole 2-oxide* **186** (54 mg, 0.08 mmol, 54%) as a yellow solid: **m.p.** 58-60 °C; R_f 0.42 (95:5, CH_2Cl_2:MeOH, UV/cerium phosphomolybdate); ν_{max} (thin film)/cm^{-1} 2921, 2305, 1754, 1616, 1530, 1437, 1260, 1169; **^{1}H NMR** (500 MHz; $CDCl_3$) δ 8.07 (2H, d, *J* 8.0, C*H*-10″,14″), 7.77 (1H, t, *J* 7.3, C*H*-12″), 7.74 (2H, d, *J* 8.2 Hz, C*H*-3′,5′), 7.69 (2H, d, *J* 8.2, C*H*-2′,6′), 7.64 (2H, t, *J* 7.8 Hz, C*H*-11″, 13″), 7.43 (1H, t, *J* 8.3 Hz, ArC*H*), 7.22-7.18 (3H, m, ArC*H* × 3), 7.13 (1H, t, *J* 2.0 Hz, C*H*-α), 7.06 (1H, dd, *J* 8.3, 2.0 Hz, C*H*-7) 6.97 (1H, dd, *J* 8.6, 2.0 Hz, C*H*-6), 6.60 (1H, ddd, *J* 9.0, 8.9, 2.0 Hz, C*H*-4), 3.81 (2H, s, 3-CH_2), 3.8 (3H, s, SOCH_3) and 2.29 (3H, s, 2-CH_3), **^{13}C NMR** (100 MHz; $CDCl_3$) δ 168.1 (quat., carboxyl), 163.4 (quat., d, *J* 249.8 Hz, C-5), 157.9 (quat., C-7″), 152.7 (quat., C-3″), 151.4 (quat., C-1″), 146.3 (quat., d, *J* 8.7 Hz, C-8), 145.6 (quat., C-4′), 141.5 (quat., C-1), 139.5 (quat., C-1′), 138.9 (quat., C-2), 137.8 (quat., C-9″), 135.9 (*C*H, C-12″), 130.7 (*C*H, C-5″), 130.4 (*C*H × 2, C-2′,6′), 130.3 (quat., C-3), 130.2 (*C*H × 2, C-11″,13″), 129.8 (*C*H × 2, C-10, C-14″), 129.5 (quat., C-9), 128.8 (*C*H, C-α), 123.9 (*C*H × 2, C-3′,5′), 123.8 (*C*H, C-7), 119.4 (*C*H, C-4″), 117.3 (*C*H, C-6″), 113.7 (*C*H_2, C-2″), 111.1 (*C*H, d, *J* 22.9 Hz, C-6), 110.6 (quat., C-8″), 106.0 (*C*H, d, *J* 24.0 Hz, C-4), 43.9 (*C*H_3, SO*C*H_3), 31.9 (*C*H_2, 3-*C*H_2) and 10.7 (*C*H_3, 2-*C*H_3), **^{19}F {^{1}H} NMR** (376 MHz; $CDCl_3$) δ -112.7 (C*F*); ***m/z*** (ES^+) 695 ($[M+Na]^+$, 100%); **HRMS** *m/z* (ES^+) calcd. for $C_{34}H_{25}FN_2NaO_8S_2$ $[M+Na]^+$ requires 695.0934, found 695.0927; **CHN** Anal. calcd. for $C_{32}H_{25}FN_2O_9S_2$: C, 60.71; H, 3.75; N, 4.16. Found C, 60.82; H, 3.88; N, 4.26.

(*Z*)-4-(3-((2-(5-Fluoro-2-methyl-1-(4-(methylsulfinyl)benzylidene)-1*H*-inden-3-yl)acetoxy)methyl)phenoxy)-3-(phenylsulfonyl)-1,2,5-oxadiazole 2-oxide, 185



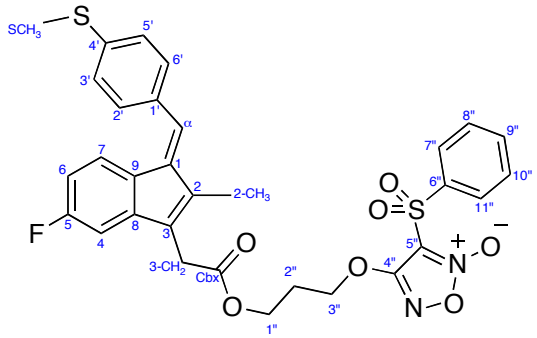
Following general procedure A with sulindac **112** (53 mg, 0.15 mmol), 4-(3-(hydroxymethyl)phenoxy)-3-(phenylsulfonyl)-1,2,5-oxadiazole 2-oxide **171** (50 mg, 0.15 mmol) purification by flash chromatography, eluting with dichloromethane and methanol (99.5:0.5), furnished *(Z)-4-(3-((2-(5-fluoro-2-methyl-1-(4-(methylsulfinyl)benzylidene)-1H-inden-3-yl)acetoxy)methyl)phenoxy)-3-(phenylsulfonyl)*-1,2*,5-oxadiazole 2-oxide* **185** (67 mg, 0.10 mmol, 68%) as a yellow solid. **m.p.** 86-84 °C; $\boldsymbol{R_f}$ 0.50 (95:5 CH_2Cl_2:MeOH, UV/cerium phosphomolybdate);); $\boldsymbol{\nu_{max}}$ (thin film)/cm^{-1} 2921, 1734, 1614, 1533, 1491, 1447, 1257, 1164, 1048; **^{1}H NMR** (400 MHz; $CDCl_3$) δ 8.10 (2H, d, *J* 8.5 Hz, C*H*-10″,14″), 7.80 (1H, tt, *J* 8.5, 1.2 Hz, C*H*-12″), 7.73 (2H, d, *J* 8.5 Hz, C*H*-3′,5′), 7.68-7.63 (4H, m, ArC*H* × 4), 7.42 (1H, t, *J* 7.9, ArC*H*), 7.28-7.24 (3H, m, ArC*H* × 3), 7.17 (1H, s, C*H*-α), 7.16 (1H, dd, *J* 8.4, 5.3, C*H*-7), 6.86 (1H, dd, *J* 8.9, 2.5, C*H*-6), 6.54 (1H, ddd, *J* 9.0, 8.9, 2.5 Hz, C*H*-4), 5.18 (2H, s, CH_2-α′), 3.64 (2H, s, 3-CH_2), 2.82 (3H, s, SOCH_3) and 2.21 (3H, s, 2-CH_3); **^{13}C NMR** (100 MHz; $CDCl_3$) δ 169.9 (quat., carboxyl), 163.4 (quat., d, *J* 247.9 Hz, C-5), 158.3 (quat., C-7″), 152.3 (quat., C-3″), 146.6 (quat., d, *J* 8.4 Hz, C-8), 145.5 (quat., C-4′), 141.5 (quat., C-1), 139.2 (quat., C-2), 138.5 (quat., C-1″), 138.2 (quat., C-1′), 137.9 (quat., C-9″), 135.9 (*C*H, C-12″), 131.4 (quat., C-3), 130.3 (*C*H × 2, C-3′,5′), 130.2 (*C*H, C-5″), 129.8 (*C*H × 2, C-10″,14″), 129.5 (quat., C-9), 128.5 (*C*H, C-α), 128.7 (*C*H × 2, C-11″,13″), 126.2 (*C*H, C-6″), 123.9 (*C*H × 2, C-2′,6′), 123.7 (*C*H, d, *J* 9.4 Hz, C-7), 119.6 (*C*H, C-2″), 119.4 (C-4″), 110.8 (*C*H, d, *J* 22.5 Hz, C-6), 110.7 (quat., C-8″), 106.1 (*C*H, d, *J* 24.0 Hz, C-4), 65.8 (CH_2, C-α′), 43.9 (CH_3, $SOCH_3$), 31.8 (CH_2, 3-CH_2) and 10.6 (CH_3, 3-CH_3); **^{19}F {^{1}H} NMR** (376 MHz; $CDCl_3$) δ -113.4 (C*F*); ***m/z*** (ES^+) 709 ($[M+Na]^+$, 100%); **HRMS** *m/z* (ES^+) calcd. for $C_{35}H_{27}FN_2NaO_8S_2$ $[M+Na]^+$ requires 709.1091, found 709.1084; **CHN** Anal. calcd. for $C_{35}H_{27}FN_2O_8S_2$: C, 61.21; H, 3.96; N, 4.08; Found C, 61.35; H, 4.07; N, 4.15.

(*Z*)-4-(2-(2-(5-Fluoro-2-methyl-1-(4-(methylthio)benzylidene)-1*H*-inden-3-yl)acetoxy)ethoxy)-3-(phenylsulfonyl)-1,2,5-oxadiazole 2-oxide, 187



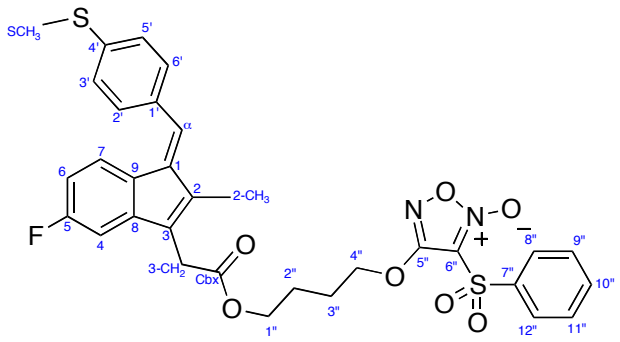
Following general procedure A with sulindac sulfide **115** (60 mg, 0.18 mmol) and 4-(2-hydroxyethoxy)-3-(phenylsulfonyl)-1,2,5-oxadiazole 2-oxide **174** (50 mg, 0.18 mmol), purification by flash chromatography, eluting with dichloromethane, furnished *(Z)-4-(2-(2-(5-fluoro-2-methyl-1-(4-(methylthio)benzylidene)-1H-inden-3-yl)acetoxy)ethoxy)-3-(phenylsulfonyl)*-1,*2,5-oxadiazole 2-oxide* **187** (39 mg, 0.08 mmol, 46%) as a yellow solid: **m.p.** 115-118 °C; $\boldsymbol{R_f}$ 0.47 (95:5, CH_2Cl_2:MeOH, UV/cerium phosphomolybdate); $\boldsymbol{\nu_{max}}$ (thin film)/cm^{-1} 2921, 1741, 1614, 1545, 1449, 1360, 1262, 1162, 1147, 825; **^{1}H NMR** (400 MHz; $CDCl_3$) δ 8.05 (2H, d, *J* 8.2 Hz, C*H*-6″,10″), 7.73 (1H, tt, 2H, d, *J* 8.2, 6.8 Hz, C*H*-8″), 7.57 (2H, t, *J* 8.2 Hz, C*H*-7″,9″), 7.44 (2H, d, *J* 8.2 Hz, C*H*-3′,5′), 7.38 (1H, dd, *J* 8.3, 5.2 Hz, C*H*-7), 7.29 (2H, d, *J* 8.2 Hz, C*H*-2′,6′), 7.17 (1H, s, C*H*-α), 6.87 (1H, dd, *J* 8.9, 2.4 Hz, C*H*-4), 6.58 (1H, ddd, *J* 9.0, 8.9, 2.4 Hz, C*H*-6), 4.66-4.62 (2H, m, CH_2-1″), 4.56-4.52 (2H, m, CH_2-2″), 3.66 (2H, s, 3-CH_2), 2.56 (3H, s, SCH_3) and 2.22 (3H, s, 2-CH_3), **^{13}C NMR** (100 MHz; $CDCl_3$) δ 170.1 (quat., carboxyl), 163.1 (quat., d, *J* 245.0, C-5), 158.7 (quat., C-3″), 146.3 (quat., d, *J* 8.5, C-8), 140.0 (quat., C-1), 139.2 (quat. × 2, C-2, C-4′), 139.8 (quat., C-1′), 138.0 (quat., C-5″), 135.6 (*C*H, C-8″), 132.9 (quat., C-3), 130.3 (*C*H, C-α), 129.9 (*C*H × 2, C-2′,6′), 129.6 (*C*H × 2, C-7″,9″), 129.9 (quat., C-9), 128.7 (*C*H × 2, C-6″,10″), 125.9 (*C*H × 2, C-3′,5′), 123.8 (*C*H, d, *J* 9.0, C-7), 110.7 (*C*H, d, *J* 22.5, C-6), 110.5 (quat., C-4″), 105.6 (*C*H, d, *J* 23.7, C-4), 68.8 (CH_2, C-2″), 61.7 (CH_2, C-1″), 31.5 (CH_2, 3-CH_2), 15.4 (CH_3, SCH_3) and 10.6 (CH_3, 2-CH_3), **^{19}F {^{1}H}** (376 MHz; $CDCl_3$) δ -114.3 (C*F*); ***m/z*** (ES^+) 631 ([M+Na]$^+$, 100%); **HRMS** *m/z* (ES^+) calcd. for $C_{30}H_{25}FN_2NaO_7S_2$, [M+Na]$^+$ requires 631.0985, found 631.0980; **CHN** Anal. calcd. for $C_{30}H_{25}FN_2O_7S_2$: C, 59.20; H, 4.14; N, 4.60. Found C, 59.30; H, 4.16; N, 4.65.

(*Z*)-4-(3-(2-(5-Fluoro-2-methyl-1-(4-(methylthio)benzylidene)-1*H*-inden-3-yl)acetoxy)propoxy)-3-(phenylsulfonyl)-1,2,5-oxadiazole 2-oxide, 188



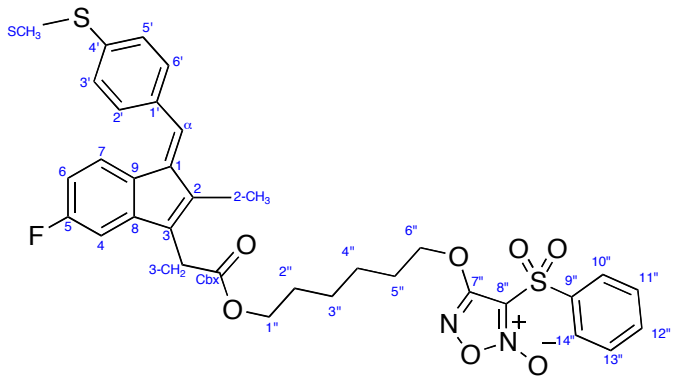
Following general procedure A with sulindac sulfide **115** (56 mg, 0.17 mmol) and 4-(3-hydroxypropoxy)-3-(phenylsulfonyl)-1,2,5-oxadiazole 2-oxide **166** (50 mg, 0.17 mmol), purification by flash chromatography, eluting with dichloromethane, furnished *(Z)-4-(3-(2-(5-fluoro-2-methyl-1-(4-(methylthio)benzylidene)-1H-inden-3-yl)acetoxy)-propoxy)-3-(phenylsulfonyl)*-1,*2,5-oxadiazole 2-oxide* **188** (47 mg, 0.08 mmol, 46%) as a yellow solid: **m.p.** 99-100 °C; $\boldsymbol{R_f}$ 0.93 (95:5, CH_2Cl_2:MeOH, UV/cerium phosphomolybdate); **H NMR** (500 MHz; $CDCl_3$) δ 8.04 (2H, d, *J* 8.3 Hz, C*H*-7″,11″), 7.73 (1H, tt, *J* 6.6, 1.6 Hz, C*H*-9″), 7.62-7.57 (2H, m, C*H*-8″,10″), 7.43 (2H, d, *J* 8.2 Hz, C*H*-3′,5′), 7.36 (1H, dd, *J* 8.2, 2.4 Hz, C*H*-7), 7.29 (2H, d, *J* 8.2 Hz, C*H*-2′,6′), 7.14 (1H, s, C*H*-α), 6.82 (1H, dd, *J* 9.0, 2.4 Hz, C*H*-4), 6.56 (1H, ddd, *J* 9.0, 8.9, 2.4 Hz, C*H*-6), 4.39 (2H, t, *J* 6.1 Hz, CH_2-1″), 4.30 (2H, t, *J* 6.1 Hz, CH_2-3″), 3.59 (2H, s, 3-CH_2), 2.55 (3H, s, SCH_3) and 2.23-2.15 (5H, m, 2-CH_3 and CH_2-2″); **^{13}C NMR** (100 MHz; $CDCl_3$) δ 170.2 (quat., carboxyl), 163.0 (quat., d, *J* 244.7 Hz, C-5), 158.7 (quat., C-4″), 146.3 (quat., d, *J* 8.6 Hz, C-8), 139.9 (quat., C-1), 139.3 (quat. × 2, C-2′, C-4′), 138.5 (quat., C-1′), 138.0 (quat., C-6″), 135.7 (*C*H, C-9″), 132.9 (quat., C-3), 130.2 (*C*H × 2, C-3′,5′), 130.5 (*C*H, C-α), 129.9 (*C*H × 2, C-2′,6′), 129.7 (*C*H × 2, C-8″,10″), 129.3 (quat., C-9), 128.5 (*C*H × 2, C-7″,11″), 123.8 (*C*H, d, *J* 9.0, C-7), 110.6 (*C*H, d, *J* 22.6 Hz, C-6), 110.4 (quat., C-5″), 105.6 (*C*H, d, *J* 23.8 Hz, C-4), 67.7 (CH_2, C-3″), 60.7 (CH_2, C-1″), 31.8 (CH_2, 3-CH_2), 27.8 (CH_2, C-2″), 15.4 (CH_3, SCH_3) and 10.6 (CH_3, 2-CH_3); **^{19}F {^{1}H} NMR** (376 MHz; $CDCl_3$) δ -114.4 (C*F*); ***m/z*** (ES^+) 645 ($[M+Na]^+$, 100%); **HRMS** *m/z* (ES^+) calcd. for $C_{31}H_{27}FN_2NaO_7S_2$ $[M+Na]^+$ requires 645.1141, found 645.1143; **CHN** Anal. calcd. for $C_{31}H_{27}FN_2O_7S_2$: C, 59.79; H, 4.37; N, 4.50. Found C, 59.84; H, 4.41; N, 4.61.

(*Z*)-4-(4-(2-(5-fluoro-2-methyl-1-(4-(methylthio)benzylidene)-1*H*-inden-3-yl)acetoxy)butoxy)-3-(phenylsulfonyl)-1,2,5-oxadiazole 2-oxide, 189



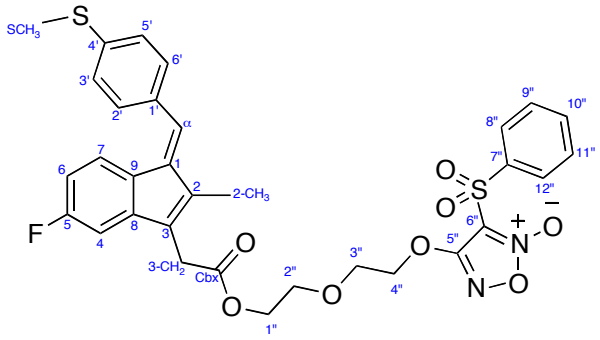
Following general procedure A with sulindac sulfide **115** (50 mg, 0.15 mmol) and 4-(4-hydroxybutoxy)-3-(phenylsulfonyl)-1,2,5-oxadiazole 2-oxide **167** (50 mg, 0.15 mmol), purification by flash chromatography, eluting with dichloromethane and methanol (99.5:0.5), furnished *(Z)-4-(4-(2-(5-fluoro-2-methyl-1-(4-(methylthio)benzylidene)-1H-inden-3-yl)acetoxy)butoxy)-3-(phenylsulfonyl)*-1,*2,5-oxadiazole 2-oxide* **189** (30 mg, 0.05 mmol, 32%) as a yellow solid: **m.p.** 68-70 °C; R_f 0.88 (95:5, CH_2Cl_2:MeOH, UV/cerium phosphomolybdate); ν_{max} (thin film)/cm^{-1} 2600, 1738, 1605, 1555, 1498, 1450, 1362, 1250, 1165, 1110; **^{1}H NMR** (500 MHz; $CDCl_3$) δ 8.04 (2H, d, *J* 8.2 Hz, C*H*-8″,12″), 7.73 (1H, t, *J* 7.5 Hz, C*H*-10″), 7.59 (2H, t, *J* 7.5 Hz, C*H*-9″,11″), 7.44 (2H, d, *J* 8.2 Hz, C*H*-3′,5′), 7.37 (1H, dd, *J* 8.5, 5.3 Hz, C*H*-7), 7.27 (2H, t, *J* 8.3 Hz, C*H*-2′,6′), 7.15 (1H, s, C*H*-α), 6.90 (1H, dd, *J* 9.0, 2.3 Hz, C*H*-4), 6.58 (1H, ddd, *J* 9.0, 9.0, 2.3 Hz, C*H*-6), 4.39 (2H, t, *J* 6.0 Hz, CH_2-1″), 4.20 (2H, t, *J* 6.2 Hz, CH_2-4″), 3.59 (2H, s, 3-CH_2), 2.55 (3H, s, SCH_3), 2.22 (3H, s, 2-CH_3), 1.93-1.87 (2H, m, CH_2-2) and 1.84-1.78 (2H, m, CH_2-3); **^{13}C NMR** (100 MHz; $CDCl_3$) δ 170.4 (quat., carboxyl), 163.7 (quat., d, *J* 245.1 Hz, C-5), 158.9 (quat., C-5″), 146.5 (quat., d, *J* 8.8 Hz, C-8), 139.9 (quat., C-1), 139.2 (quat., C-4′), 138.5 (quat., C-7″), 138.0 (*C*H, C-10″), 135.6 (quat., C-2), 132.8 (quat., C-3), 130.6 (quat., C-1′), 130.1 (*C*H, C-α), 130.0 (*C*H × 2, C-9″,11″), 129.9 (*C*H × 2, C-2′, C-5′), 129.7 (*C*H × 2, C-8″,12″), 128.5 (quat., C-9), 125.9 (*C*H × 2, C-3′,5′), 123.7 (*C*H, d, *J* 8.9, C-7), 112.0 (quat., C-6″), 110.6 (*C*H, d, *J* 22.3 Hz, C-6), 105.7 (*C*H, d, *J* 23.7 Hz, C-4), 70.9 (CH_2, C-4″), 64.2 (CH_2, C-1″), 31.9 (CH_2, 3-CH_2), 25.1 (CH_2, C-3″), 25.0 (CH_2, C-2″), 15.4 (CH_3, SCH_3) and 10.6 (CH_3, 2-CH_3); **^{19}F {^{1}H} NMR** (376 MHz; $CDCl_3$) δ -113.9 (C*F*); ***m/z*** (ES^+) 658 ($[M+Na]^+$, 100%); **HRMS** *m/z* (ES^+) calcd. for $C_{32}H_{29}FN_2O_7NaS_2$ $[M+Na]^+$ requires 659.1298, found 659.1302; **CHN** Anal. calcd. for $C_{32}H_{29}FN_2O_8S_2$: C, 60.36; H, 4.59; N, 4.40. Found C, 60.42; H, 4.65; N, 4.48.

(*Z*)-4-((6-(2-(5-Fluoro-2-methyl-1-(4-(methylthio)benzylidene)-1*H*-inden-3-yl)acetoxy)hexyl)oxy)-3-(phenylsulfonyl)-1,2,5-oxadiazole 2-oxide, 190



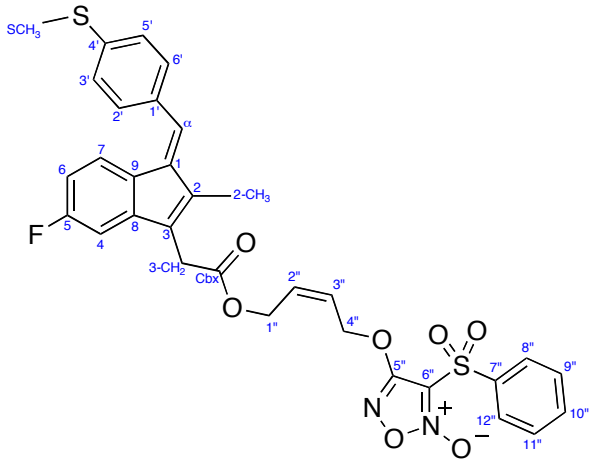
Following general procedure A with sulindac sulfide **115** (50 mg, 0.15 mmol) and 4-((6-hydroxyhexyl)oxy)-(phenylsulfonyl)-1,2,5-oxadiazole 2-oxide **168** (50 mg, 0.15 mmol), purification by flash chromatography, eluting with dichloromethane furnished *(Z)-4-((6-(2-(5-fluoro-2-methyl-1-(4-(methylthio)benzylidene)-1H-inden-3-yl)acetoxy)-hexyl)oxy)-3-(phenylsulfonyl)*-1,*2,5-oxadiazole 2-oxide* **190** (79 mg, 0.12 mmol, 80%) as a yellow solid: **m.p.** 67-77 °C; $\boldsymbol{R_f}$ 0.40 (CH_2Cl2, UV/cerium phosphomolybdate); $\boldsymbol{\nu_{max}}$ (thin film)/cm^{-1} 2616, 1732, 1611, 1550, 1488, 1456, 1356, 1257, 1169; **^{1}H NMR** (400 MHz; $CDCl_3$) δ, 8.05 (2H, d, *J* 8.5 Hz, C*H*-10″,14″), 7.73 (1H, tt, *J* 7.5, 1.7, C*H*-12″), 7.60 (2H, t, *J* 7.5 Hz, C*H*-11″,13″), 7.44 (2H, d, *J* 8.1 Hz, C*H*-3′,5′), 7.38 (1H, dd, *J* 8.4, 5.3 Hz, C*H*-7), 7.29 (2H, d, *J* 8.4 Hz, C*H*-2′,6′), 7.15 (1H, s, C*H*-α), 6.91 (1H, dd, *J* 9.0, 2.5 Hz, C*H*-4), 6.59 (1H, ddd, *J* 9.0, 8.9, 2.5 Hz, C*H*-6), 4.38 (2H, t, *J* 6.5 Hz, CH_2-1″), 4.14 (2H, t, *J* 6.5 Hz, CH_2-6″), 3.58 (2H, s, 3-CH_2), 2.55 (3H, s, SCH_3), 2.22 (3H, s, 2-CH_3), 1.86-1.79 (2H, m, CH_2-2″), 1.70-1.63 (2H, m, CH_2-5″) and 1.49-1.34 (4H, m, CH_2 × 2, CH_2-3″, CH_2-4″); **^{13}C NMR** (100 MHz; $CDCl_3$) δ 170.3 (quat., carboxyl), 163.3 (quat., d, *J* 246.6 Hz, C-5), 159.0 (quat., C-7″), 146.8 (quat., d, *J* 8.6 Hz, C-8), 145.4 (quat., C-4′), 141.6 (quat., C-1), 139.7 (quat., C-1′), 138.2 (quat., C-2), 138.1 (quat., C-9″), 135.6 (*C*H, C-12″), 131.9 (quat., C-3), 130.3 (*C*H × 2, C-2′,6′), 129.7 (*C*H × 2, C-10″,14″), 129.5 (quat., C-9), 128.5 (*C*H × 2, C-11″,13″), 128.3 (*C*H, C-α), 125.9 (*C*H × 2, C-3′,5′), 123.7 (*C*H, d, *J* 9.0 Hz, C-7), 110.8 (*C*H, d, *J* 23.0 Hz, C-6), 110.5 (quat., C-8″), 106.2 (*C*H, d, *J* 23.8 Hz, C-4), 71.4 (CH_2, C-6″), 64.9 (CH_2, C-1″), 43.9 (CH_3, $SOCH_3$), 31.9 (CH_2, 3-CH_2), 28.4 (CH_2, C-5″), 28.3 (CH_2, C-2″), 25.5 (CH_2, C-4″), 25.2 (CH_2, C-3″) and 10.6 (CH_3, 3-CH_3); **^{19}F {^{1}H} NMR** (376 MHz; $CDCl_3$) δ -114.4 (C*F*); ***m/z*** (ES^+) 687 ($[M+Na]^+$, 100%); **HRMS** *m/z* (ES^+) calcd. for $C_{34}H_{33}FN_2NaO_7S_2$ $[M+Na]^+$ requires 687.1611, found 687.1615; **CHN** Anal. calcd. for $C_{34}H_{33}FN_2O_7S_2$: C, 61.43; H, 5.00; N, 4.21. Found C, 61.51; H, 5.11; N, 4.24.

(*Z*)-4-(2-(2-(2-(5-Fluoro-2-methyl-1-(4-(methylthio)benzylidene)-1*H*-inden-3-yl)acetoxy)ethoxy)ethoxy)-3-(phenylsulfonyl)-1,2,5-oxadiazole 2-oxide, 191



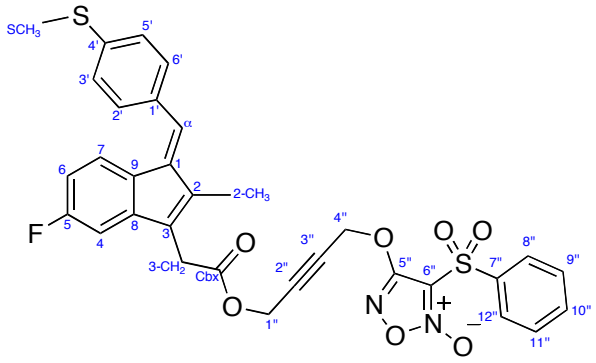
Following general procedure A with sulindac sulfide **115** (50 mg, 0.15 mmol) and 4-(2-(3-hydroxyethoxy)ethoxy)-3-(phenylsulfonyl)-1,2,5-oxadiazole 2-oxide **175** (50 mg, 0.15 mmol), purification by flash chromatography, eluting with dichloromethane, furnished *(Z)-4-(2-(2-(2-(5-fluoro-2-methyl-1-(4-(methylthio)benzylidene)-1H-inden-3-yl)acetoxy)ethoxy)ethoxy)-3-(phenylsulfonyl)*-1,*2,5-oxadiazole 2-oxide* **191** (41 mg , 0.06 mmol, 41%) as a yellow solid: **m.p.** 50-53 °C; R_f 0.83 (95:5, CH_2Cl_2:MeOH, UV/cerium phosphomolybdate); ν_{max} (thin film)/cm^{-1} 2931, 1729, 1609, 1550, 1491, 1449, 1638, 1260, 1088; **^{1}H NMR** (300 MHz; $CDCl_3$) δ 8.05 (2H, d, *J* 8.2 Hz, C*H*-8″,12″), 7.72 (1H, tt, *J* 7.6, 1.1 Hz, C*H*-10″), 7.58 (2H, t, *J* 7.7 Hz, C*H*-9″,11″), 7.42 (2H, d, *J* 8.1 Hz, C*H*-3′,5′), 7.35 (1H, dd, *J* 8.4, 5.3 Hz, C*H*-7), 7.28 (2H, d, *J* 8.1 Hz, C*H*-2′,6′), 7.14 (1H, s, C*H*-α), 6.91 (1H, dd, *J* 9.0, 2.4 Hz, C*H*-4), 6.56 (1H, ddd, *J* 9.0, 8.8, 2.4, C*H*-6), 4.50-4.48 (2H, m, CH2-1″), 4.32-4.30 (2H, m, CH_2-4″), 3.84-3.82 (2H, m, CH_2-2″), 3.79-3.76 (2H, m, CH_2-3), 3.61 (2H, s, 3-CH_2), 2.55 (3H, s, SCH_3) and 2.20 (3H, s, 2-CH_3); **^{13}C NMR** (100 MHz; $CDCl_3$) δ 170.4 (quat., carboxyl), 163.1 (quat., d, *J* 249.0 Hz, C-5), 158.9 (quat., C-5″), 146.5 (quat., d, *J* 9.3 Hz, C-8), 140.0 (quat., C-1), 139.2 (quat. × 2, C-2, C-4′), 138.6 (quat., C-1′), 138.1 (quat., C-7″), 135.6 (*C*H, C-10″), 132.9 (quat., C-3), 130.0 (quat., C-9), 129.9 (*C*H × 2, C-2′,6′), 129.6 (*C*H × 2, C-9″,11″), 128.6 (*C*H × 2, C-8″,12″), 128.4 (*C*H, C-α), 125.9 (*C*H × 2, C-3′,5′), 123.7 (*C*H, d, *J* 8.7, C-7), 110.6 (*C*H, d, *J* 23.4 Hz, C-6), 110.5 (quat., C-6″), 105.9 (*C*H, d, *J* 23.9 Hz, C-4), 70.5 (*C*H_2, C-4″), 69.4 (*C*H_2, C-2″), 68.4 (*C*H_2, C-5″), 64.0 (*C*H_2, C-1″), 15.4 (*C*H_3, SCH_3), 31.8 (*C*H_2, 3-CH_2) and 10.6 (*C*H_3, 2-CH_3); **^{19}F {^{1}H} NMR** (376 MHz; $CDCl_3$) δ -114.2 (C*F*); ***m/z*** (ES^+) 675 ($[M+Na]^+$, 100%); **HRMS** *m/z* (ES^+) calcd. for $C_{32}H_{29}FN_2NaO_8S_2$ $[M+Na]^+$ requires 675.1247, found 675.1239; **CHN** Anal. calcd. for $C_{32}H_{29}FN_2O_8S_2$: C, 58.88; H, 4.48; N, 4.29. Found C, 58.98; H, 4.52; N, 4.33.

4-(((*Z*)-4-(2-((*Z*)-5-Fluoro-2-methyl-1-(4-(methylthio)benzylidene)-1*H*-inden-3-yl)acetoxy)but-2-en-1-yl)oxy)-3-(phenylsulfonyl)-1,2,5-oxadiazole 2-oxide, 192



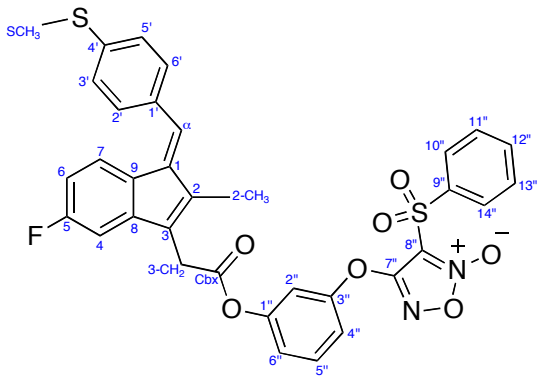
Following general procedure A with sulindac sulfide **115** (60 mg, 0.17 mmol) and (*Z*)-4-((4-hydroxybut-2-en-1-yl)oxy)-3-(phenylsulfonyl)-1,2,5-oxadiazole 2-oxide **170** (50 mg, 0.17 mmol), purification by flash chromatography, eluting with dichloromethane and hexane (90:10), furnished *4-(((Z)-4-(2-((Z)-5-fluoro-2-methyl-1-(4-(methylthio)benzylidene)-1H-inden-3-yl)acetoxy)but-2-en-1-yl)oxy)-3-(phenylsulfonyl)*-1,*2,5-oxadiazole 2-oxide* **192** (56 mg, 0.09 mmol, 55%) as a yellow solid **m.p.** 84-86 °C; $\boldsymbol{R_f}$ 0.92 (95:5, CH_2Cl_2:MeOH, UV/cerium phosphomolybdate); $\boldsymbol{\nu_{max}}$ (thin film)/cm^{-1} 2921, 1732, 1606, 1545, 1491, 1449, 1309; **^{1}H NMR** (500 MHz; $CDCl_3$) δ 8.05 (2H, d, *J* 8.3 Hz, C*H*-8″,12″), 7.73 (1H, tt, *J* 7.5, 1.3 Hz, C*H*-10″), 7.60 (2H, t, *J* 8.1 Hz, C*H*-9″,11″), 7.44 (2H, d, *J* 8.2 Hz, C*H*-3′,5′), 7.36 (1H, dd, *J* 8.4, 5.3, C*H*-7), 7.28 (2H, d, *J* 8.4 Hz, C*H*-2′,6′), 7.15 (1H, s, C*H*-α), 6.86 (1H, dd, *J* 9.0, 2.4 Hz, C*H*-6), 6.58 (1H, ddd, *J* 9.0, 8.9, 2.4 Hz, C*H*-4), 5.94-5.83 (2H, m, C*H*-2″, 3″), 5.01 (2H, d, *J* 5.0 Hz, CH_2-1″), 4.75 (2H, d, *J* 5.1 Hz, CH_2-4″), 3.59 (2H, s, 3-CH_2), 2.55 (3H, s, SCH_3) and 2.20 (3H, s, 2-CH_3); **^{13}C NMR** (100 MHz; $CDCl_3$) δ 170.1 (quat., carboxyl), 163.1 (quat., d, *J* 244.6 Hz, C-5), 158.5 (quat., C-5″), 146.3 (quat., d, *J* 8.8 Hz, C-8), 139.9 (quat., C-1), 139.2 (quat. × 2, C-2, C-4′), 138.7 (quat., C-1′), 138.0 (quat., C-7″), 135.7 (*C*H, C-10″), 132.9 (quat., C-3), 130.2 (*C*H × 2, C-2′,6′), 130.4 (*C*H, C-α), 129.8 (*C*H × 2, C-9″,11″), 129.7 (*C*H, C-3″), 129.7 (quat., C-9), 128.6 (*C*H × 2, C-8″,12″), 126.1 (*C*H, C-2″), 125.9 (*C*H × 2, C-3′,5′), 123.8 (*C*H, d, *J* 8.8 Hz, C-7), 110.6 (*C*H, d, *J* 22.3 Hz, C-6), 110.5 (quat., C-6″), 105.7 (*C*H, d, *J* 24.1 Hz, C-4), 66.6 (*C*H_2, C-4″), 60.5 (C-1″), 31.7 (*C*H_2, 3-CH_2), 15.4 (*C*H_3, SCH_3) and 10.6 (*C*H_3, 2-CH_3); **^{19}F {^{1}H} NMR** (376 MHz; $CDCl_3$) δ -114.3 (C*F*); ***m/z*** (ES^+) 657 ($[M+Na]^+$, 100%); **HRMS** *m/z* (ES^+) calcd. for $C_{32}H_{27}FN_2NaO_7S_2$, $[M+Na]^+$ requires 657.1141, found 657.1135; **CHN** Anal. calcd. for $C_{32}H_{27}FN_2O_7S_2$: C, 60.56; H, 4.29; N, 4.41. Found C, 60.70; H, 4.40; N, 4.51.

(*Z*)-4-((4-(2-(5-Fluoro-2-methyl-1-(4-(methylthio)benzylidene)-1*H*-inden-3-yl)acetoxy)but-2-yn-1-yl)oxy)-3-(phenylsulfonyl)-1,2,5-oxadiazole 2-oxide, 193



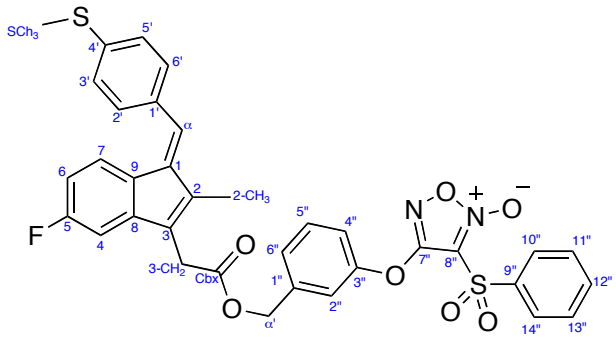
Following general procedure A with sulindac sulfide **115** (60 mg, 0.17 mmol), 4-((4-hydroxybut-2-yn-1-yl)oxy)-3-(phenylsulfonyl)-1,2,5-oxadiazole 2-oxide **169** (50 mg, 0.17 mmol), purification by flash chromatography, eluting with dichloromethane, furnished *(Z)-4-((4-(2-(5-fluoro-2-methyl-1-(4-(methylthio)benzylidene)-1H-inden-3-yl)acetoxy)but-2-yn-1-yl)oxy)-3-(phenylsulfonyl)*-1,*2,5-oxadiazole 2-oxide* **193** (57 mg, 0.09 mmol, 56%) as a yellow solid: **m.p.** 79-80 °C; $\boldsymbol{R_f}$ 0.94 (95:5 CH_2Cl_2:MeOH, UV/cerium phosphomolybdate); $\boldsymbol{\nu_{max}}$ (thin film)/cm^{-1} 2911, 1739, 1611, 1545, 1488, 1454, 1360, 1260, 1169, 822; **^{1}H NMR** (400 MHz; $CDCl_3$) δ 8.07 (2H, d, *J* 8.5 Hz, C*H*-8″,12″), 7.75 (1H, tt, *J* 7.5, 1.1 Hz, C*H*-10″), 7.62 (2H, t, *J* 7.5 Hz, C*H*-9″,11″), 7.45 (2H, d, *J* 8.3 Hz, C*H*-3′,5′), 7.38 (1H, dd, *J* 8.4, 5.2 Hz, C*H*-7), 7.30 (2H, d, *J* 8.4 Hz, C*H*-2′,6′), 7.17 (1H, s, C*H*-α), 6.89 (1H, dd, *J* 9.0, 2.4 Hz, C*H*-6), 6.59 (1H, ddd, *J* 9.0, 9.0, 2.4 Hz, C*H*-4), 5.10 (2H, t, *J* 1.6 Hz, CH_2-1″), 4.78 (2H, t, *J* 1.6 Hz, CH_2-4), 3.63 (2H, s, 3-CH_2), 2.55 (3H, s, SCH_3) and 2.22 (3H, s, 2-CH_3); **^{13}C NMR** (100 MHz; $CDCl_3$) δ, 169.6 (quat., carboxyl), 163.1 (quat., d, *J* 246.0 Hz, C-5), 157.9 (quat., C-5″), 146.6 (quat., d, *J* 8.7 Hz, C-8), 139.9 (quat., C-1), 139.3 (quat., C-4′), 138.9 (quat., C-1′), 139.3 (quat., C-2), 137.8 (quat., C-7″), 135.7 (*C*H, C-10″), 132.0 (quat., C-3), 130.3 (*C*H, C-α), 129.9 (*C*H × 2, C-2′,6′), 129.8 (quat., C-9), 129.7 (*C*H × 2, C-8″,12″), 128.6 (*C*H × 2, C-3‴, C-5‴), 125.9 (*C*H × 2, C-3′,5′), 123.7 (*C*H, d, *J* 9.0 Hz, C-7), 110.7 (*C*H, d, *J* 22.6 Hz, C-6), 110.6 (quat., C-6″), 105.7 (*C*H, d, *J* 24.1 Hz, C-4), 83.8 (quat., C-3″), 78.8 (quat., C-2″), 58.6 (CH_2, C-4″), 52.4 (CH_2, C-1″), , 31.5 (CH_2, 3-CH_2), 15.4 (CH_3, SCH_3) and 10.6 (CH_3, 3-CH_3); **^{19}F {^{1}H} NMR** (376 MHz; $CDCl_3$) δ, -114.3 (C*F*); ***m/z*** (ES^+) 655 ($[M+Na]^+$, 100%); **HRMS** *m/z* (ES^+) calcd. for $C_{32}H_{25}FN_2NaO_7S_2$ $[M+Na]^+$ requires 655.0985, found 655.0980; **CHN** Anal. calcd. for $C_{32}H_{25}FN_2O_7S_2$: C, 60.75; H, 3.98; N, 4.43. Found C, 60.84; H, 4.06; N, 4.50.

(*Z*)-4-(3-(2-(5-Fluoro-2-methyl-1-(4-(methylthio)benzylidene)-1*H*-inden-3-yl)acetoxy)phenoxy)-3-(phenylsulfonyl)-1,2,5-oxadiazole 2-oxide, 195



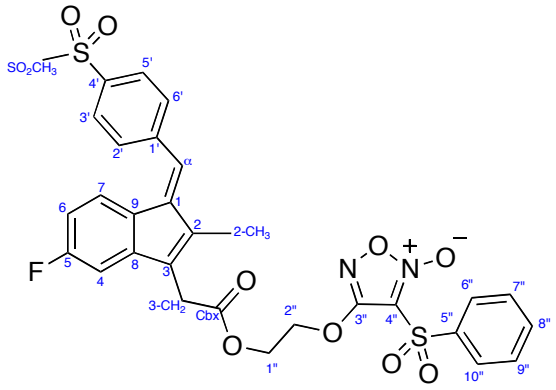
Following general procedure A with sulindac sulfide **115** (51 mg, 0.15 mmol), 4-(3-hydroxyphenoxy)-3-(phenylsulfonyl)-1,2,5-oxadiazole 2-oxide **172** (50 mg, 0.15 mmol), purification by flash chromatography, eluting with dichloromethane and hexane (90:10), furnished *(Z)-4-(3-(2-(5-fluoro-2-methyl-1-(4-(methylthio)benzylidene)-1H-inden-3-yl)acetoxy)phenoxy)-3-(phenylsulfonyl)*-1,2,*5-oxadiazole 2-oxide* **195** (48 mg, 0.07 mmol, 49%) as a yellow solid: **m.p.** 59-61 °C; ***R*$_f$** 0.71 (CH_2Cl2, UV/cerium phosphomolybdate); **ν_{max}** (thin film)/cm^{-1} 2070, 1750, 1620, 1530, 1490, 1395, 1156, 1120; **^{1}H NMR** (300 MHz; $CDCl_3$) δ 8.06 (2H, d, *J* 8.4 Hz, C*H*-10″,14″), 7.76 (1H, tt, *J* 7.3, 1.3 Hz, C*H*-12″), 7.62 (2H, t, *J* 8.0 Hz, C*H*-11″,13″), 7.46 (2H, d, *J* 8.0 Hz, C*H*-3′,5′), 7.43-7.38 (2H, m, ArC*H* × 2), 7.30 (2H, d, *J* 8.4 Hz, C*H*-2′,6′), 7.21-7.18 (2H, m, ArC*H* × 2), 7.11 (1H, t, *J* 2.3 Hz, ArC*H*), 7.05 (1H, ddd, *J* 8.2, 2.2, 0.9 Hz, ArC*H*), 6.96 (1H, dd, *J* 9.0, 2.4 Hz, C*H*-6), 6.62 (1H, ddd, *J* 9.0, 8.9, 2.4 Hz, C*H*-4), 3.80 (2H, s, 3-CH_2), 2.55 (3H, s, SCH_3) and 2.28 (3H, s, 2-CH_3); **^{13}C NMR** (100 MHz; $CDCl_3$) δ 168.3 (quat., carboxyl), 163.1 (quat., d, *J* 246.0 Hz, C-5), 157.9 (quat., C-7″), 152.8 (quat., C-3″), 151.5 (quat., C-1″), 146.2 (quat., d, *J* 8.7, C-8), 139.9 (quat., C-1), 139.4 (quat., C-1′), 139.2 (quat. × 2, C-2, C-4′), 137.9 (quat., C-9″), 135.9 (*C*H, C-12″), 132.8 (quat., C-3), 130.5 (*C*H, C-5″), 130.4 (*C*H, C-α), 129.9 (*C*H × 2, C-3′,5′), 129.8 (*C*H × 2, C-11″,13″), 129.6 (quat., C-9), 128.6 (*C*H × 2, C-10″, C-1″), 125.9 (*C*H × 2, C-2′,6′), 123.9 (*C*H, d, *J* 9.2, C-7), 119.9 (*C*H, C-4″), 117.2 (*C*H, C-6″), 113.7 (*C*H_2, C-2″), 112.2 (quat., C-8″) 110.8 (*C*H, d, *J* 22.6, C-6), 105.7 (*C*H, d, *J* 24.6, C-4), 31.9 (*C*H_2, 3-CH_2), 15.4 (*C*H_3, SCH_3) and 10.8 (*C*H_3, 2-CH_3); **^{19}F {^{1}H} NMR** (376 MHz; $CDCl_3$) δ -114.0 (C*F*); ***m/z*** (ES^+) 679 ($[M+Na]^+$, 100%); **HRMS** *m/z* (ES^+) calcd. for $C_{34}H_{25}FN_2NaO_7S_2$, 679.0985 found 679.0975; **CHN** Anal. calcd. for $C_{32}H_{25}FN_2O_7S_2$: C, 62.18; H, 3.84; N, 4.27. Found C, 62.29; H, 3.95; N, 4.20.

(*Z*)-4-(3-((2-(5-Fluoro-2-methyl-1-(4-(methylthio)benzylidene)-1*H*-inden-3-yl)acetoxy)methyl)phenoxy)-3-(phenylsulfonyl)-1,2,5-oxadiazole 2-oxide, 194



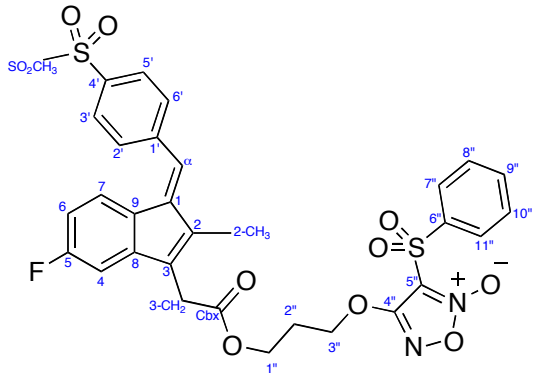
Following general procedure A with sulindac sulfide **115** (50 mg, 0.15 mmol) and 4-(3-(hydroxymethyl)phenoxy)-3-(phenylsulfonyl)-1,2,5-oxadiazole 2-oxide **171** (50 mg, 0.15 mmol) purification by flash chromatography, eluting with dichloromethane, furnished *(Z)-4-(3-((2-(5-fluoro-2-methyl-1-(4-(methylthio)benzylidene)-1H-inden-3-yl)acetoxy)methyl)phenoxy)-3-(phenylsulfonyl)*-1,2*,5-oxadiazole 2-oxide* **194** (64 mg, 0.10 mmol, 65%) as a yellow solid: **m.p.** 99-101 °C; R_f 0.56 (CH_2Cl_2, UV/cerium phosphomolybdate); ν_{max} (thin film)/cm^{-1} 2068, 1749, 1623, 1533. 1442, 1260, 1166, 1122; **^{1}H NMR** (400 MHz; $CDCl_3$) δ, 8.10 (2H, d, *J* 8.3, C*H*-10″,14″), 7.79 (1H, tt, *J* 7.5, 1.3 Hz, C*H*-12″), 7.65 (2H, t, *J* 7.8 Hz, C*H*-11″,13″), 7.45 (2H, d, *J* 8.1 Hz, C*H*-3′,5′), 7.40 (1H, d, *J* 7.8 Hz, ArC*H*), 7.37 (1H, dd, *J* 8.4, 5.2 Hz, C*H*-7), 7.31-7.29 (3H, m, ArC*H* × 3), 7.27-7.24 (2H, m, ArC*H* × 2), 7.15 (1H, s, C*H*-α), 6.86 (1H, dd, *J* 9.0, 2.4 Hz, C*H*-6), 6.56 (1H, ddd, *J* 9.0, 8.8, 2.4 Hz, C*H*-4), 5.17 (2H, s, CH_2-α′), 3.64 (2H, s, 3-CH_2), 2.62 (3H, s, SCH_3) and 2.21 (3H, s, 2-CH_3); **^{13}C NMR** (100 MHz; $CDCl_3$) δ, 170.0 (quat., carboxyl), 163.0 (quat., d, *J* 244.9 Hz, C-5), 158.3 (quat., C-7″), 152.6 (quat., C-3″), 146.3 (quat., d, *J* 8.7 Hz, C-8), 140.0 (quat., C-1), 139.2 (quat. × 2, C-2, C-4′), 138.7 (quat., C-1″), 138.3 (quat., C-1′), 137.9 (quat., C-9″), 135.8 (*C*H, C-12″), 132.9 (quat., C-3), 130.3 (*C*H, C-α), 130.2 (*C*H, C-5″), 129.9 (*C*H × 3, C-2′,6′, C-9), 129.8 (*C*H × 2, C-10″,14″), 128.6 (*C*H × 2, C-11″,13″), 126.2 (*C*H, C-6″), 125.9 (*C*H × 2, C-3′,5′), 123.7 (*C*H, d, *J* 9.0 Hz, C-7), 119.6 (*C*H, C-2″), 119.4 (C-4″), 110.7 (quat., C-8″), 110.6 (*C*H, d, *J* 22.4 Hz, C-6), 105.7 (*C*H, d, *J* 23.3 Hz, C-4), 65.7 (CH_2, C-α′), 31.8 (CH_2, 3-CH_2), 15.4 (CH_3, SCH_3) and 10.6 (CH_3, 3-CH_3); **^{19}F {^{1}H} NMR** (376 MHz; $CDCl_3$) δ -114.4 (C*F*); ***m/z*** (ES^+) 693 ($[M+Na]^+$, 100%); **HRMS** *m/z* (ES^+) calcd. for $C_{35}H_{27}FN_2NaO_7S_2$ $[M+Na]^+$ requires 693.1141, found 693.1135; **CHN** Anal. calcd. for $C_{35}H_{27}FN_2O_7S_2$: C, 62.67; H, 4.06; N, 4.18; Found C, 62.71; H, 4.15; N, 4.15.

(*Z*)-4-(2-(2-(5-Fluoro-2-methyl-1-(4-(methylsulfonyl)benzylidene)-1*H*-inden-3-yl)acetoxy)ethoxy)-3-(phenylsulfonyl)-1,2,5-oxadiazole 2-oxide, 196



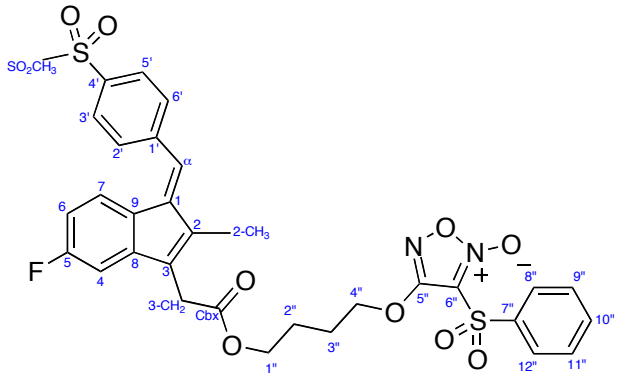
Following general procedure A with sulindac sulfone **116** (65 mg, 0.18 mmol) and 4-(2-hydroxyethoxy)-3-(phenylsulfonyl)-1,2,5-oxadiazole 2-oxide **174** (50 mg, 0.18 mmol), purification by flash chromatography, eluting with dichoromethane, furnished *(Z)-4-(2-(2-(5-fluoro-2-methyl-1-(4-(methylsulfonyl)benzylidene)-1H-inden-3-yl)acetoxy)ethoxy)-3-(phenylsulfonyl)*-1,2*,5-oxadiazole 2-oxide* **196** (22 mg, 0.04 mmol, 25%) as a yellow solid: **m.p.** 88-93 °C; $\boldsymbol{R_f}$ 0.84 (95:5, CH_2Cl_2:MeOH, UV/cerium phosphomolybdate); $\boldsymbol{\nu_{max}}$ (thin film)/cm^{-1} 2926, 1737, 1611, 1550, 1491, 1456, 1400, 1360, 1556, 1085; **^{1}H NMR** (400 MHz; $CDCl_3$) δ 8.04 (2H, d, *J* 8.2 Hz, C*H*-6″,10″), 8.00 (2H, d, *J* 8.2 Hz, C*H*-3′,5′), 7.74 (1H, t, *J* 7.5, C*H*-8″), 7.70 (2H, d, *J* 8.2 Hz, C*H*-2′,6′), 7.58-7.55 (2H, m, C*H*-7″,9″), 7.15 (1H, s, C*H*-α), 7.10 (1H, dd, *J* 8.7, 5.1, C*H*-7), 6.88 (1H, dd, *J* 9.0, 2.1 Hz, C*H*-4), 6.57 (1H, ddd, *J* 9/0, 9.0, 2.1 Hz, C*H*-6), 4.65-4.63 (2H, m, CH_2-1″), 4.57-4.54 (2H, m, CH_2-2″), 3.66 (2H, s, 3-CH_2), 3.14 (3H, s, SO_2CH_3) and 2.23 (3H, s, 2-CH_3); **^{13}C NMR** (100 MHz; $CDCl_3$) δ 169.9 (quat., carboxyl), 163.5 (quat., d, *J* 245.5 Hz, C-5), 158.5 (quat., C-3″), 146.7 (quat., d, *J* 9.0 Hz, C-8), 142.5 (quat., C-4′), 142.2 (quat., C-1′), 139.9 (quat., C-1), 138.6 (quat., C-2), 137.9 (quat., C-5″), 135.7 (*C*H, C-8″), 131.7 (quat., C-3), 130.2 (*C*H × 2, C-2′,6′), 129.7 (*C*H × 2, C-7″,9″), 129.3 (quat., C-9), 128.7 (*C*H × 2, C-3′,5′), 127.9 (*C*H, C-α), 127.7 (*C*H × 2, C-6″,10″), 123.8 (*C*H, d, *J* 9.3 Hz, C-7), 111.0 (*C*H, d, *J* 22.7 Hz, C-6), 110.5 (quat., C-4″), 106.2 (*C*H, d, *J* 24.1, C-4), 67.7 (*C*H_2, C-2″), 61.8 (*C*H_2, C-1″), 44.5 (*C*H_3, SO_2CH_3), 31.6 (*C*H_2, 3-CH_2) and 10.6 (*C*H_3, 2-CH_3); **^{19}F {^{1}H} NMR** (376 MHz; $CDCl_3$) δ -112.8 (C*F*); ***m/z*** (ES^+) 663 ($[M+Na]^+$, 100%); **HRMS** *m/z* (ES^+) calcd. for $C_{30}H_{25}FN_2NaO_9S_2$ $[M+Na]^+$ requires 663.0883, found 663.0889; **CHN** Anal. calcd. for $C_{30}H_{25}FN_2O_9S_2$: C, 56.24; H, 3.93; N, 4.37. Found C, 56.36; H, 4.01; N, 4.45.

(*Z*)-4-(3-(2-(5-Fluoro-2-methyl-1-(4-(methylsulfonyl)benzylidene)-1*H*-inden-3-yl)acetoxy)propoxy)-3-(phenylsulfonyl)-1,2,5-oxadiazole 2-oxide, 197



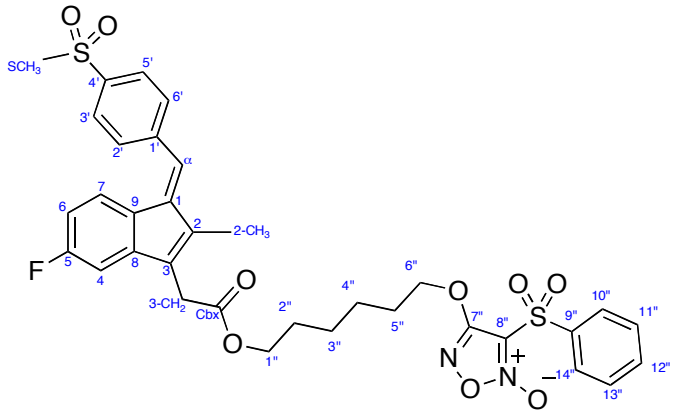
Following general procedure A with sulindac sulfone **116** (62 mg, 0.17 mmol) and 4-(3-hydroxypropoxy)-3-(phenylsulfonyl)-1,2,5-oxadiazole 2-oxide **166** (50 mg, 0.17 mmol), purification by flash chromatography, eluting dichoromethane, furnished *(Z)-4-(3-(2-(5-fluoro-2-methyl-1-(4-(methylsulfonyl)benzylidene)-1H-inden-3-yl)acetoxy)propoxy)-3-(phenylsulfonyl)*-1,*2,5-oxadiazole 2-oxide* **197** (85 mg, 0.13 mmol, 77%) as a yellow solid: **m.p.** 108-110 °C; $\boldsymbol{R_f}$ 0.82 (95:5, CH_2Cl_2:MeOH, UV/cerium phosphomolybdate); $\boldsymbol{\nu_{max}}$ (thin film)/cm^{-1} 2926, 1729, 1611, 1547, 1491, 1449, 1257, 1166, 1088; **^{1}H NMR** (500 MHz; $CDCl_3$) δ 8.06-7.99 (4H, m, C*H*-3′,5′,7″,11″), 7.77-7.69 (3H, m, C*H*-2,6′,9″), 7.64 (2H, t, *J* 8.0 Hz, C*H*-8″,10″), 7.13 (1H, s, C*H*-α), 7.09 (1H, dd, *J* 8.4, 5.2 Hz, C*H*-7), 6.84 (1H, dd, *J* 8.8, 2.4 Hz, C*H*-4), 6.55 (1H, ddd, *J* 8.9, 8.8, 2.4 Hz, C*H*-6), 4.40 (2H, t, *J* 6.1 Hz, CH_2-1″), 4.32 (2H, t, *J* 6.0 Hz, CH_2-3″), 3.59 (2H, s, 3-CH_2), 3.14 (3H, s, SO_2CH_3) and 2.24-2.16 (5H, m, 2-CH_3 and CH_2-2″); **^{13}C NMR** (100 MHz; $CDCl_3$) δ 169.9 (quat., carboxyl), 163.0 (quat., d, *J* 247.0 Hz, C-5), 158.7 (quat., C-4″), 146.8 (quat., C-8), 142.4 (quat., C-4′), 142.2 (quat., C-1′), 139.9 (quat., C-1), 138.2 (quat., C-2), 138.0 (quat., C-6″), 135.7 (*C*H, C-9″), 132.1 (quat., C-3), 130.3 (*C*H × 2, C-2′,6′), 129.7 (*C*H × 2, C-8″,10″), 129.3 (quat., C-9), 128.6 (*C*H × 2, C-3′,5′), 127.7 (*C*H × 2, C-7″,11″), 127.4 (*C*H, C-α), 123.8 (*C*H, d, *J* 9.3 Hz, C-7), 110.0 (*C*H, d, *J* 22.7 Hz, C-6), 110.4 (quat., C-4″), 106.2 (*C*H, d, *J* 23.4, C-4), 67.7 (CH_2, C-3″), 60.9 (CH_2, C-1″), 44.5 (CH_3, SO_2CH_3), 31.7 (CH_2, 3-CH_2), 27.8 (CH_2, C-2″) and 10.2 (CH_3, 2-CH_3); **^{19}F {^{1}H} NMR** (376 MHz; $CDCl_3$) δ -112.9 (C*F*); ***m/z*** (ES^+) 677 ($[M+Na]^+$, 100%); **HRMS** *m/z* (ES^+) calcd. for $C_{31}H_{27}FN_2NaO_9S_2$ $[M+Na]^+$ requires 677.1040, found 677.1045; **CHN** Anal. calcd. for $C_{31}H_{27}FN_2O_9S_2$: C, 56.87; H, 4.16; N, 4.28. Found C, 56.92; H, 4.22; N, 4.29.

(*Z*)-4-(4-(2-(5-Fluoro-2-methyl-1-(4-(methylsulfonyl)benzylidene)-1*H*-inden-3-yl)acetoxy)butoxy)-3-(phenylsulfonyl)-1,2,5-oxadiazole 2-oxide, 198



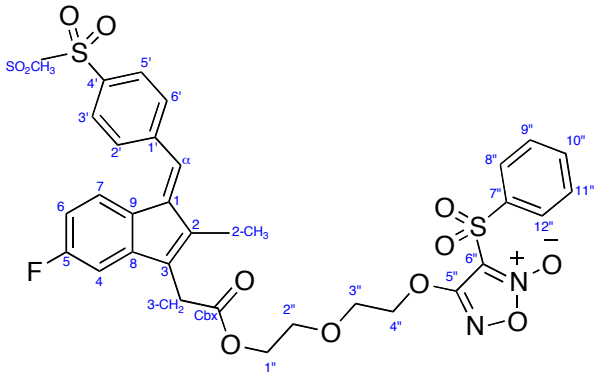
Following general procedure A with sulindac sulfone **116** (50 mg, 0.13 mmol) and 4-(4-hydroxybutoxy)-3-(phenylsulfonyl)-1,2,5-oxadiazole 2-oxide **167** (50 mg, 0.15 mmol) purification by flash chromatography, eluting with dichloromethane and methanol (99.5:0.5), furnished *(Z)-4-(4-(2-(5-fluoro-2-methyl-1-(4-(methylsulfonyl)benzylidene) -1H-inden-3-yl)acetoxy)butoxy)-3-(phenylsulfonyl)*-1,*2,5-oxadiazole 2-oxide* **198** (23 mg, 0.04 mmol, 26%) as a yellow solid: **m.p.** 64-68 °C; $\boldsymbol{R_f}$ 0.72 (95:5, CH_2Cl_2:MeOH, UV/cerium phosphomolybdate); $\boldsymbol{\nu_{max}}$ (thin film)/cm^{-1} 2931, 1733, 1613, 1562, 1471, 1440, 1355, 1300, 1140, 1088; **^{1}H NMR** (500 MHz; $CDCl_3$) δ 8.02 (2H, d, *J* 8.3 Hz, C*H*-8″,12″), 7.93 (2H, d, *J* 8.3 Hz, C*H*-3′,5′), 7.67 (1H, t, *J* 7.5 Hz, C*H*-10″), 7.62 (2H, d, *J* 8.2 Hz, C*H*-2′,6′), 7.53 (2H, t, *J* 8.0 Hz, C*H*- 9″,11″), 7.07 (1H, s, C*H*-α), 7.03 (1H, dd, *J* 8.4, 5.1 Hz, C*H*-7), 6.83 (1H, dd, *J* 8.8, 2.3 Hz, C*H*-4), 6.50 (1H, ddd, *J* 8.9, 8.8, 2.3 Hz, C*H*-6), 4.33 (2H, t, *J* 6.1 Hz, CH_2-1″), 4.14 (2H, t, *J* 6.2 Hz, CH_2-4), 3.53 (2H, s, 3-CH_2), 3.07 (3H, s, SO_2CH_3), 2.15 (3H, s, 2-CH_3), 1.87-1.82 (2H, m, CH_2-2″) and 1.78-1.73 (2H, m, CH_2-3″); **^{13}C NMR** (100 MHz; $CDCl_3$) δ 169.1 (quat., carboxyl), 162.4 (quat., d, *J* 246.4 Hz, C-5), 157.9 (quat., C-5″), 145.8 (quat., d, *J* 8.8 Hz, C-8), 142.5 (quat., C-4′), 141.5 (quat., C-1′), 141.4 (quat., C-1), 139.7 (quat., C-7″), 138.8 (quat., C-2), 134.6 (*C*H, C-4‴), 131.2 (quat., C-3), 127.5 (*C*H, C-α), 130.2 (*C*H × 2, C-2′,6′), 129.7 (*C*H × 2, C-8″,12″), 128.6 (*C*H × 2, C-3′,5′), 128.3 (quat., C-9), 127.5 (*C*H × 2, C-7″,11″), 122.7 (*C*H, d, *J* 9.1 Hz, C-7), 111.9 (quat., C-6″) 109.9 (*C*H, d, *J* 22.4 Hz, C-6), 105.3 (*C*H, d, *J* 23.9 Hz, C-4), 69.8 (*C*H_2, C-4″), 63.3 (*C*H_2, C-1″), 43.4 (*C*H_3, SO_2CH_3), 30.9 (*C*H_2, 3-CH_2), 24.2 (*C*H_2, C-3″), 24.0 (*C*H_2, C-2″) and 9.5 (*C*H_3, 2-CH_3); **^{19}F {^{1}H} NMR** (376 MHz; $CDCl_3$) δ -114.4 (C*F*); ***m/z*** (ES^+) 691 ($[M+Na]^+$, 100%); **HRMS** *m/z* (ES^+) calcd. for $C_{32}H_{29}FN_2O_9NaS_2$ $[M+Na]^+$ requires 691.1196, found 691.1200; **CHN** Anal. calcd. for $C_{32}H_{29}FN_2O_9S_2$: C, 57.48; H, 4.37; N, 4.19. Found C, 57.63; H, 4.46; N, 4.22.

(*Z*)-4-((6-(2-(5-Fluoro-2-methyl-1-(4-(methylsulfonyl)benzylidene)-1*H*-inden-3-yl)acetoxy)hexyl)oxy)-3-(phenylsulfonyl)-1,2,5-oxadiazole 2-oxide, 199



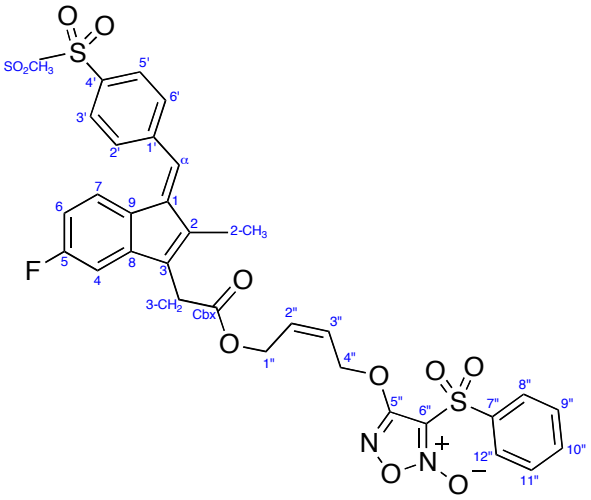
Following general procedure A with sulindac sulfone **116** (54 mg, 0.15 mmol) and 4-((6-hydroxyhexyl)oxy)-3-(phenylsulfonyl)-1,2,5-oxadiazole 2-oxide **168** (50 mg, 0.15 mmol), purification by flash chromatography, eluting with dichloromethane, furnished *(Z)-4-((6-(2-(5-fluoro-2-methyl-1-(4-(methylsulfonyl)benzylidene)-1H-inden-3-yl)acetoxy)hexyl)oxy)-3-(phenylsulfonyl)*-1,2*,5-oxadiazole 2-oxide* **199** (72 mg, 0.10 mmol, 71%) as a yellow solid: **m.p.** 60-63 °C; $\boldsymbol{R_f}$ 0.33 (100% CH_2Cl_2, UV/cerium phosphomolybdate); $\boldsymbol{\nu_{max}}$ (thin film)/cm^{-1} 2926, 1734, 1614, 1552, 1469, 1449, 1358, 1309, 1149; **^{1}H NMR** (400 MHz; $CDCl_3$) δ 8.05 (2H, d, *J* 8.6 Hz, C*H*-10″,14″), 8.01 (2H, d, *J* 8.4 Hz, C*H*-3′,5′), 7.75 (1H, tt, *J* 7.6, 1.7 Hz, C*H*-12″), 7.70 (2H, d, *J* 8.0 Hz, C*H*-3′,5′), 7.61 (2H, t, *J* 7.7 Hz, C*H*-11″,13″), 7.14 (1H, s, C*H*-α), 7.11 (1H, dd, *J* 8.4, 5.1 Hz, C*H*-7), 6.91 (1H, dd, *J* 8.9, 2.4 Hz, C*H*-4), 6.57 (1H, ddd, *J* 9.0, 8.9, 2.4 Hz, C*H*-6), 4.39 (2H, t, *J* 6.6 Hz, CH_2-1″), 4.15 (2H, t, *J* 6.6 Hz, CH_2-6), 3.58 (2H, s, 3-CH_2), 3.14 (3H, s, SO_2CH_3), 2.22 (3H, s, 2-CH_3), 1.89-1.80 (2H, m, CH_2-2″), 1.72-1.65 (2H, m, CH_2-5″) and 1.50-1.36 (4H, m, CH_2 × 2, CH_2-3″, CH_2-4″); **^{13}C NMR** (100 MHz; $CDCl_3$) δ, 170.2 (quat., carboxyl), 163.4 (quat., d, *J* 247.3 Hz, C-5), 159.0 (quat., C-7″), 146.9 (quat., d, *J* 8.8 Hz, C-8), 142.5 (quat., C-4′), 142.3 (quat., C-1′), 139.9 (quat., C-1), 138.1 (quat. × 2, C-2, C-9″), 135.6 (*C*H, C-12″), 132.4 (quat., C-3), 130.2 (*C*H × 2, C-3′,5′), 129.7 (*C*H × 2, C-10″,14″), 129.4 (quat., C-9), 128.5 (*C*H × 2, C-2′,6′), 127.6 (*C*H × 2, C-11″,13″), 127.3 (*C*H, C-α), 123.7 (*C*H, d, *J* 9.0, C-7), 110.9 (*C*H, d, *J* 22.9 Hz, C-6), 110.5 (quat., C-8″), 106.4 (*C*H, d, *J* 24.0 Hz, C-4), 71.4 (CH_2, C-6″), 64.9 (CH_2, C-1″), 44.5 (CH_3, SO_2CH_3), 31.9 (CH_2, 3-CH_2), 28.4 (CH_2, C-5″), 28.3 (CH_2, C-2″), 25.5 (CH_2, C-4″), 25.2 (CH_2, C-3″) and 10.5 (CH_3, 3-CH_3); **^{19}F {^{1}H} NMR** (376 MHz; $CDCl_3$) δ -113.0 (C*F*); ***m/z*** (ES^+) 719 ($[M+Na]^+$, 100%); **HRMS** *m/z* (ES^+) calcd. for $C_{34}H_{33}FN_2NaO_9S_2$ $[M+Na]^+$ requires 719.1509, found 719.1501; **CHN** Anal. calcd. for $C_{34}H_{33}FN_2O_9S_2$: C, 58.61; H, 4.77; N, 4.02. Found C, 58.72; H, 4.84; N, 4.08.

(*Z*)-4-(2-(2-(2-(5-Fluoro-2-methyl-1-(4-(methylsulfonyl)benzylidene)-1*H*-inden-3-yl)acetoxy)ethoxy)ethoxy)-3-(phenylsulfonyl)-1,2,5-oxadiazole 2-oxide, 200



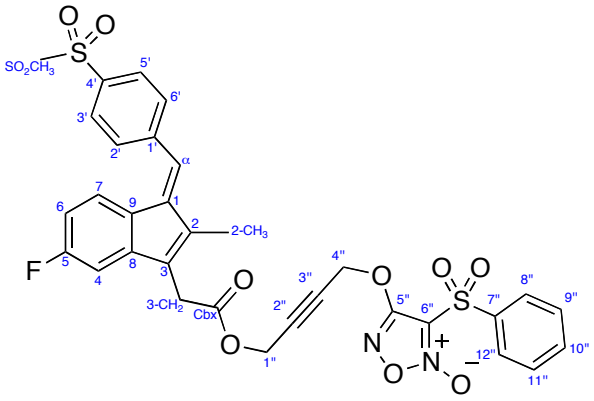
Following general procedure A with sulindac sulfone **116** (55 mg, 0.15 mmol) and 4-(2-(3-hydroxyethoxy)ethoxy)-3-(phenylsulfonyl)-1,2,5-oxadiazole 2-oxide **175** (50 mg, 0.15 mmol), purification by flash chromatography, eluting with dichloromethane, furnished *(Z)-4-(2-(2-(5-fluoro-2-methyl-1-(4-(methylsulfonyl)benzylidene)-1H-inden-3-yl)acetoxy)ethoxy)ethoxy)-3-(phenylsulfonyl)*-1,*2,5-oxadiazole 2-oxide* **200** (63.2, 0.09 mmol, 62%) as a yellow solid: **m.p.** 60-63 °C; $\boldsymbol{R_f}$ 0.67 (95:5, CH_2Cl_2:MeOH, UV/cerium phosphomolybdate); $\boldsymbol{\nu_{max}}$ (thin film)/cm^{-1} 2926, 1727, 1611, 1552, 1459, 1365, 1309, 1260, 1156, 1088; **^{1}H NMR** (300 MHz; $CDCl_3$) δ 8.09-8.04 (2H, m, C*H*-8″,12″), 8.00 (2H, d, *J* 8.4 Hz, C*H*-3′,5′), 7.76-7.53 (5H, m, C*H*-2′,6′,9″,10″,11″), 7.12 (1H, s, C*H*-α), 7.09 (1H, dd, *J* 8.4, 5.1 Hz, C*H*-7), 6.92 (1H, dd, *J* 8.9, 2.4 Hz, C*H*-6), 6.55 (1H, ddd, *J* 9.0, 8.9, 2.4 Hz, C*H*-4), 4.53-4.49 (2H, m, CH_2-1″), 4.34-4.31 (2H, m, CH_2-4″), 3.87-3.84 (2H, m, CH_2-2), 3.80-3.77 (2H, m, CH_2-3), 3.62 (2H, s, 3-CH_2), 3.14 (3H, s, SO_2CH_3) and 2.20 (3H, s, 2-CH_3); **^{13}C NMR** (100 MHz; $CDCl_3$) δ 170.1 (quat., carboxyl), 163.4 (quat., d, *J* 247.2 Hz, C-5), 158.79 (quat., C-5″), 146.9 (quat., d, *J* 8.9 Hz, C-8), 142.3 (quat., C-4′), 142.3 (quat., C-1), 139.9 (quat., C-1′), 138.3 (quat., C-2), 138.0 (quat., C-7″), 135.6 (*C*H, C-10″), 132.2 (quat., C-3), 130.2 (*C*H × 2, C-2′,6′), 129.7 (*C*H × 2, C-9″,11″), 129.4 (quat., C-9), 127.3 (*C*H, C-α), 127.6 (*C*H × 2, C-8″,12″), 123.9 (*C*H × 2, C-3′,5′), 123.7 (*C*H, d, *J* 8.9 Hz, C-7), 110.9 (*C*H, d, *J* 22.8 Hz, C-6), 110.5 (quat., C-6″), 106.2 (*C*H, d, *J* 24.4 Hz, C-4), 70.5 (CH_2, C-4″), 69.4 (CH_2, C-2″), 68.3 (CH_2, C-5″), 64.1 (CH_2, C-1″), 44.5 (CH_3, SO_2CH_3), 31.7 (CH_2, 3-CH_2) and 10.5 (CH_3, 2-CH_3); **^{19}F {^{1}H} NMR** (376 MHz; $CDCl_3$) δ -113.0 (C*F*); ***m/z*** (ES^+) 707 ($[M+Na]^+$, 100%); **HRMS** *m/z* (ES^+) calcd. for $C_{32}H_{29}FN_2NaO_{10}S_2$ $[M+Na]^+$ requires 707.1145, found 707.1139; **CHN** Anal. calcd. for $C_{32}H_{29}FN_2O_{10}S_2$: C, 56.13; H, 4.27; N, 4.09. Found C, 56.20; H, 4.35; N, 4.20.

4-(((*Z*)-4-(2-((*Z*)-5-fluoro-2-methyl-1-(4-(methylsulfonyl)benzylidene)-1*H*-inden-3-yl)acetoxy)but-2-en-1-yl)oxy)-3-(phenylsulfonyl)-1,2,5-oxadiazole 2-oxide, 201



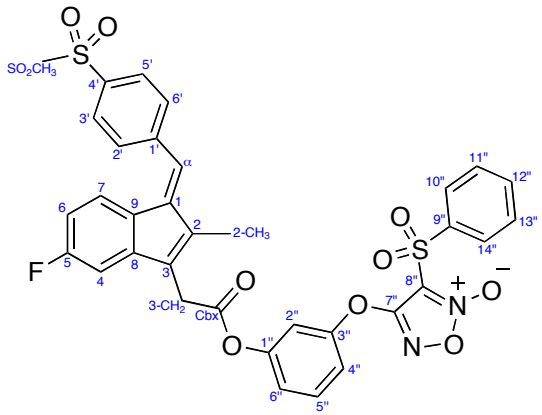
Following general procedure A with sulindac sulfone **116** (60 mg, 0.17 mmol) and (*Z*)-4-((4-hydroxybut-2-en-1-yl)oxy)-3-(phenylsulfonyl)-1,2,5-oxadiazole 2-oxide **170** (50 mg, 0.17 mmol) purification by flash chromatography, eluting with dichloromethane, furnished *4-(((Z)-4-(2-((Z)-5-fluoro-2-methyl-1-(4-(methylsulfonyl)benzylidene)-1H-inden-3-yl)acetoxy)but-2-en-1-yl)oxy)-3-(phenylsulfonyl)*-1,2,*5-oxadiazole 2-oxide* **201** (96 mg, 0.15 mmol, 93%) as a yellow solid: **m.p.** 109-110 °C; R_f 0.86 (95:5, CH_2Cl_2:MeOH, UV/cerium phosphomolybdate); ν_{max} (thin film)/cm^{-1} 2921, 1732, 1611, 1547, 1447, 1365, 1260, 1166, 1083; **^{1}H NMR** (500 MHz; $CDCl_3$) δ 8.06-7.99 (4H, m, C*H*-3′,5′,8″,12″), 7.78-7.68 (3H, m, C*H*-2′,6′,10″), 7.60 (2H, t, *J* 8.0 Hz, C*H*-9″,11″), 7.14 (1H, s, C*H*-α), 7.10 (1H, dd, *J* 8.4, 5.1 Hz, C*H*-7), 6.87 (1H, dd, *J* 8.8, 2.4 Hz, C*H*-6), 6.57 (1H, ddd, *J* 8.9, 8.8, 2.4 Hz, C*H*-4), 5.96-5.84 (2H, m, C*H*-2″, 3″), 5.01 (2H, d, *J* 5.2 Hz, CH_2-1″), 4.77 (2H, d, *J* 5.3 Hz, CH_2-4″), 3.60 (2H, s, 3-CH_2), 3.14 (3H, s, SO_2CH_3) and 2.21 (3H, s, 2-CH_3); **^{13}C NMR** (100 MHz; $CDCl_3$) δ 169.8 (quat., carboxyl), 163.5 (quat., d, *J* 245.8 Hz, C-5), 158.5 (quat., C-5″), 146.7 (quat., d, *J* 8.7 Hz, C-8), 142.5 (quat., C-4′), 142.3 (quat., C-1′), 139.9 (quat., C-1), 138.3 (quat., C-2), 137.9 (quat., C-7″), 135.7 (*C*H, C-10″), 132.0 (quat., C-3), 130.2 (*C*H × 2, C-3′,5′), 129.9 (*C*H, C-3″), 129.3 (quat., C-9), 129.7 (*C*H × 2, C-9″,11″), 128.6 (*C*H × 2, C-8″,12″), 128.4 (*C*H × 2, C-2′,6′), 127.4 (*C*H, C-α), 126.2 (*C*H, C-2″), 123.8 (*C*H, d, *J* 9.2 Hz, C-7), 111.0 (*C*H, d, *J* 22.2 Hz, C-6), 110.6 (quat., C-6″), 106.3 (*C*H, d, *J* 23.7 Hz, C-4), 66.5 (*C*H_2, C-4″), 60.6 (C-1″), 44.5 (*C*H_3, SO_2CH_3), 31.7 (*C*H_2, 3-CH_2) and 10.6 (*C*H_3, 2-CH_3); **^{19}F {^{1}H} NMR** (376 MHz; $CDCl_3$) δ -112.8 (C*F*); ***m/z*** (ES^+) 689 ($[M+Na]^+$, 100%); **HRMS** *m/z* (ES^+) calcd. for $C_{32}H_{27}FN_2NaO_9S_2$ $[M+Na]^+$ requires 689.1040, found 689.1046; **CHN** Anal. calcd. for $C_{32}H_{27}FN_2O_9S_2$: C, 57.65; H, 4.08; N, 4.20. Found C, 57.72; H, 4.13; N, 4.26.

(*Z*)-4-((4-(2-(5-Fluoro-2-methyl-1-(4-(methylsulfonyl)benzylidene)-1*H*-inden-3-yl)acetoxy)but-2-yn-1-yl)oxy)-3-(phenylsulfonyl)-1,2,5-oxadiazole 2-oxide, 202



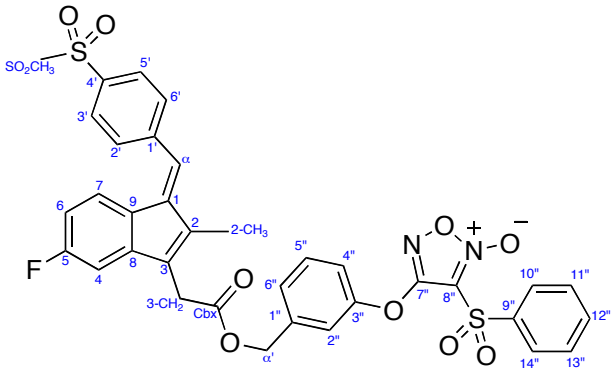
Following general procedure A with sulindac sulfone **116** (55 mg, 0.17 mmol), 4-((4-hydroxybut-2-yn-1-yl)oxy)-3-(phenylsulfonyl)-1,2,5-oxadiazole 2-oxide **169** (50 mg, 0.17 mmol), purification by flash chromatography, eluting with dichloromethane, furnished (*Z*)-4-((4-(2-(5-fluoro-2-methyl-1-(4-(methylsulfonyl)benzylidene)-1H-inden-3-yl)acetoxy)but-2-yn-1-yl)oxy)-3-(phenylsulfonyl)-1,2,5-oxadiazole 2-oxide **202** (32 mg, 0.05 mmol, 32%) as a yellow solid: **m.p.** 99-100 °C; R_f 0.80 (95:5 CH_2Cl_2:MeOH); ν_{max} (thin film)/cm^{-1} 2921, 1739, 1609, 1547, 1449, 1358, 1309, 1169, 1144; **^{1}H NMR** (400 MHz; $CDCl_3$) δ 8.07 (2H, d, *J* 8.5 Hz, C*H*-8″,12″), 8.01 (2H, d, *J* 8.4 Hz, C*H*-3′,5′), 7.89-7.59 (5H, m, C*H*-2′,6′,9″,10″,11″), 7.15 (1H, s, C*H*-α), 7.10 (1H, dd, *J* 5.2, 8.4, C*H*-7), 6.88 (1H, dd, *J* 8.9, 2.4 Hz, C*H*-6), 6.57 (1H, ddd, *J* 9.0, 8.9, 2.4, C*H*-4), 5.10 (2H, t, *J* 1.6 Hz, CH_2-1″), 4.78 (2H, t, *J* 1.6 Hz, CH_2-4), 3.63 (2H, s, 3-CH_2), 3.14 (3H, s, SO_2CH_3) and 2.22 (3H, s, 2-CH_3); **^{13}C NMR** (100 MHz; $CDCl_3$) δ 169.3 (quat., carboxyl), 163.4 (quat., d, *J* 246.2 Hz, C-5), 157.9 (quat., C-5″), 146.6 (quat., d, *J* 9.0 Hz, C-8), 142.4 (quat., C-4′), 142.2 (quat., C-1′), 139.9 (quat., C-1), 138.5 (quat., C-2), 137.8 (quat., C-7″), 135.7 (*C*H, C-10″), 131.6 (quat., C-3), 130.2 (*C*H × 2, C-3′,5′), 129.7 (*C*H × 2, C-8″,12″), 128.7 (*C*H × 2, C-2′,6′), 129.3 (quat., C-9), 127.6 (*C*H × 2, C-9″,11″), 127.5 (*C*H, C-α), 123.8 (*C*H, d, *J* 9.0 Hz, C-7), 111.0 (*C*H, d, *J* 22.8 Hz, C-6), 110.6 (quat., C-6″), 106.3 (*C*H, d, *J* 24.3 Hz, C-4), 83.7 (quat., C-3″), 78.9 (quat., C-2″), 60.4 (*C*H_2, C-4″), 58.5 (*C*H_2, C-1″), 44.5 (*C*H_3, SO_2CH_3), 31.4 (*C*H_2, 3-CH_2) and 10.6 (*C*H_3, 3-CH_3); **^{19}F {^{1}H} NMR** (376 MHz; $CDCl_3$) δ -112.9 (C*F*); ***m/z*** (ES^+) 687 ($[M+Na]^+$, 100%); **HRMS** *m/z* (ES^+) calcd. for $C_{32}H_{25}FN_2NaO_9S_2$ $[M+Na]^+$ requires 687.0883, found 687.0878; **CHN** Anal. calcd. for $C_{32}H_{25}FN_2O_9S_2$: C, 57.82; H, 3.79; N, 4.21. Found C, 58.00; H, 3.84; N, 4.26.

(*Z*)-4-(3-(2-(5-Fluoro-2-methyl-1-(4-(methylsulfonyl)benzylidene)-1*H*-inden-3-yl)acetoxy)phenoxy)-3-(phenylsulfonyl)-1,2,5-oxadiazole 2-oxide, 204



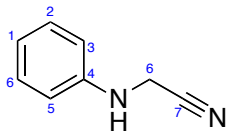
Following general procedure A with sulindac sulfone **116** (56 mg, 0.15 mmol) and 4-(3-hydroxyphenoxy)-3-(phenylsulfonyl)-1,2,5-oxadiazole 2-oxide **172** (50 mg, 0.15 mmol), purification by flash chromatography, eluting with dichloromethane and hexane (90:10), furnished *(Z)-4-(3-(2-(5-fluoro-2-methyl-1-(4-(methylsulfonyl)benzylidene)-1H-inden-3-yl)acetoxy)-phenoxy)-3-(phenylsulfonyl)*-1,2,5-*oxadiazole 2-oxide* **204** (52 mg, 0.08 mmol, 50%) as a yellow solid: **m.p.** 62-64 °C; R_f 0.79 (95:5, CH_2Cl_2:MeOH, UV/cerium phosphomolybdate); ν_{max} (thin film)/cm^{-1} 2921, 1732, 1609, 1491, 1449, 1309, 1147, 1048; **^{1}H NMR** (500 MHz; $CDCl_3$) δ 8.07 (2H, d, *J* 8.3, C*H*-10″,14″), 8.02 (2H, d, *J* 8.3 Hz, C*H*-3′,5′), 7.77 (1H, t, *J* 7.5 Hz, C*H*-12″), 7.72 (2H, d, *J* 8.0 Hz, C*H*-2′,6′), 7.63 (2H, t, *J* 7.9 Hz, C*H*-11″,13″), 7.43 (1H, d, *J* 8.3 Hz, ArC*H*), 7.20 (1H, dd, *J* 8.3, 2.3 Hz, ArC*H*), 7.18 (1H, s, C*H*-α), 7.15-7.12 (2H, m, ArC*H* × 2), 7.06 (1H, dd, *J* 8.2, 1.9 Hz, C*H*-7), 6.97 (1H, dd, *J* 8.8, 2.4 Hz, C*H*-6), 6.61 (1H, ddd, *J* 9.0, 8.8, 2.4 Hz, C*H*-4), 3.81 (2H, s, 3-CH_2), 3.14 (3H, s, SO_2CH_3) and 2.28 (3H, s, 2-CH_3); **^{13}C NMR** (100 MHz; $CDCl_3$) δ 168.0 (quat., carboxyl), 163.5 (quat., d, *J* 246.0 Hz, C-5), 157.9 (quat., C-7″), 152.7 (quat., C-3″), 151.3 (quat., C-1″), 146.5 (quat., d, *J* 8.7 Hz, C-8), 142.3 (quat., C-4′), 142.2 (quat., C-1′), 140.0 (quat., C-1), 138.8 (quat., C-2), 137.8 (quat., C-9″), 135.9 (*C*H, C-12″), 131.3 (quat., C-3), 130.4 (*C*H, C-5″), 130.2 (*C*H × 2, C-3′,5′), 129.3 (quat., C-9), 129.8 (*C*H × 2, C-11″,13″), 128.6 (*C*H × 2, C-2′,6′), 127.8 (*C*H, C-α), 127.7 (*C*H × 2, C-10″,14″), 123.9 (*C*H, d, *J* 9.2, C-7), 119.8 (*C*H, C-4″), 117.3 (*C*H, C-6″), 113.7 (*C*H_2, C-2″), 111.2 (*C*H, d, *J* 22.8 Hz, C-6), 110.6 (quat., C-8″), 106.2 (*C*H, d, *J* 23.9 Hz, C-4), 44.5 (*C*H_3, SO_2CH_3), 31.9 (*C*H_2, 3-CH_2) and 10.7 (*C*H_3, 2-CH_3); **^{19}F {^{1}H} NMR** (376 MHz; $CDCl_3$) δ -113.3 (C*F*); ***m/z*** (ES^+) 711 ($[M+Na]^+$, 100%); **HRMS** *m/z* (ES^+) calcd. for $C_{34}H_{25}FN_2NaO_9S_2$ $[M+Na]^+$ requires 711.0883, found 711.0878, **CHN** Anal. calcd. for $C_{32}H_{25}FN_2O_9S_2$: C, 59.29; H, 3.66; N, 4.07; N, 4.27. Found C, 59.38; H, 3.70; N, 4.02.

(*Z*)-4-(3-((2-(5-Fluoro-2-methyl-1-(4-(methylsulfonyl)benzylidene)-1*H*-inden-3-yl)acetoxy)methyl)phenoxy)-3-(phenylsulfonyl)-1,2,5-oxadiazole 2-oxide, 203



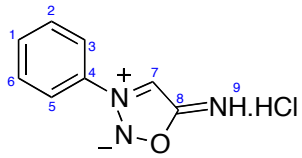
Following general procedure A with sulindac sulfone **116** (54 mg, 0.15 mmol) and 4-(3-(hydroxymethyl)phenoxy)-3-(phenylsulfonyl)-1,2,5-oxadiazole 2-oxide **171** (50 mg, 0.15 mmol) purification by flash chromatography, eluting with dichloromethane and methanol (99.5:0.5), furnished *(Z)-4-(3-((2-(5-fluoro-2-methyl-1-(4-(methylsulfonyl)benzylidene)-1H-inden-3-yl)acetoxy)methyl)phenoxy)-3-(phenyl-sulfonyl)*-1,2,*5-oxadiazole 2-oxide* **203** (69 mg, 0.10 mmol, 66%) as a yellow solid: **m.p.** 119-120 °C; R_f 0.83 (95:5 CH_2Cl_2:MeOH, UV/cerium phosphomolybdate); ν_{max} (thin film)/cm^{-1} 3068, 2926, 1734, 1616, 1533, 1442, 1306, 1159, 1085; **^{1}H NMR** (400 MHz; $CDCl_3$) δ 8.11 (2H, d, *J* 8.5 Hz, C*H*-10″,12″), 8.03 (2H, d, *J* 8.3 Hz, C*H*-3′,5′), 7.82 (1H, tt, *J* 7.5, 1.6 Hz, C*H*-12″), 7.72 (2H, d, *J* 8.3 Hz, ArC*H* × 2), 7.67 (2H, t, *J* 7.9 Hz, C*H*-11″,13″), 7.44 (1H, t, *J* 7.9 Hz, ArC*H*), 7.27-7.25 (3H, m, ArC*H* × 3), 7.16 (1H, s, C*H*-α), 7.12 (1H, dd, *J* 5.1, 8.4 Hz, C*H*-7), 6.88 (1H, dd, *J* 8.9, 2.4 Hz, C*H*-6), 6.56 (1H, ddd, *J* 9.0, 8.9, 2.4 Hz, C*H*-4), 5.20 (2H, s, CH_2- α′), 3.66 (2H, s, 3-CH_2), 3.17 (3H, s, SO_2CH_3) and 2.22 (3H, s, 2-CH_3); **^{13}C NMR** (100 MHz; $CDCl_3$) δ 169.8 (quat., carboxyl), 163.3 (quat., d, *J* 247.5 Hz, C-5), 158.3 (quat., C-7″), 152.6 (quat., C-3″), 146.6 (quat., d, *J* 8.5 Hz, C-8), 142.4 (quat., C-4′), 142.3 (quat., C-1′), 139.9 (quat., C-1), 138.4 (quat., C-1″), 138.2 (quat., C-2), 137.9 (quat., C-9″), 135.9 (*C*H, C-12″), 131.9 (quat., C-3), 130.2 (*C*H × 3, C-3′,5′, C-5″), 129.8 (*C*H × 2, C-10″,14″), 129.3 (quat., C-9), 128.6 (*C*H, C-α), 128.6 (*C*H × 2, C-11″,13″), 128.4 (*C*H × 2, C-2′,6′), 126.2 (*C*H, C-6″), 123.7 (*C*H, d, *J* 9.0 Hz, C-7), 119.6 (*C*H, C-2″), 119.3 (C-4″), 110.9 (*C*H, d, *J* 22.6 Hz, C-6), 110.7 (quat., C-8″), 106.3 (*C*H, d, *J* 22.7, C-4), 65.8 (CH_2, C-α′), 44.5 (CH_3, SO_2CH_3), 31.8 (CH_2, 3-CH_2) and 10.6 (CH_3, 3-CH_3); **^{19}F {^{1}H} NMR** (376 MHz; $CDCl_3$) δ -112.9 (C*F*); ***m/z*** (ES^+) 725 ($[M+Na]^+$, 100%); **HRMS** *m/z* (ES^+) calcd. for $C_{35}H_{27}FN_2NaO_9S_2$ $[M+Na]^+$ requires 725.1040, found 725.1038; **CHN** Anal. calcd. for $C_{35}H_{27}FN_2O_9S_2$: C, 59.82; H, 3.87; N, 3.99; Found C, 60.00; H, 3.99; N, 4.06.

2-(Phenylamino)acetonitrile, 208



Bromoacetonitrile (6.27 g, 3.64 mL, 52.0 mmol), potassium carbonate (8.3 g, 60.1 mmol) and sodium iodide (3.7 g, 24.7 mmol) were added to a solution of freshly distilled aniline **207** (4.55 g, 4.5 mL, 48.9 mmol) in dry acetonitrile (100 mL) and the suspension stirred at room temperature overnight in the absence of light. The suspension was filtered and the filtrate partitioned between diethyl ether (100 mL) and distilled water (100 mL). The organic layer was separated and washed with distilled water (50 mL) and brine (50 mL), dried over Na_2SO_4, filtered and the solvent removed under reduced pressure to yield 3-(phenylamino)acetonitrile **208** (5.62 g, 42.6 mmol, 87%) as a colourless oil which crystallised on standing, which was used without any further purification: **m.p.** 40-42 °C, [Lit.[400] 40 °C]; **^{1}H NMR** (300 MHz; $CDCl_3$) δ 7.24-7.17 (2H, m, C*H*-3,5), 6.81 (1H, tt, *J* 8.4, 1.1 Hz, C*H*-1), 6.65-6.61 (2H, m, C*H*-2,6) and 3.99-3.95 (3H, m, CH_2, N*H*); **^{13}C NMR** (100 MHz; $CDCl_3$) δ 145.3 (quat., C-4), 129.8 (*C*H × 2, C-2, 6), 120.2 (*C*H, C-1), 117.3 (quat., C-7), 113.8 (*C*H × 2, C-3, 5) and 32.8 (*C*H_2, C-6); ***m/z*** (ES$^+$) 155 ([M+Na]$^+$, 100%). The data were in agreement with the literature values.[400]

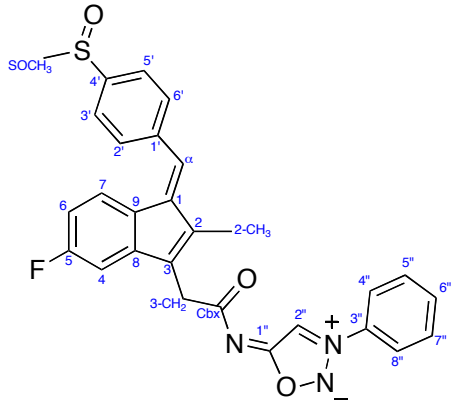
3-Phenylsydononimine hydrochloride, 206[94]



Isopentyl nitrite (1.41 g, 1.6 mL, 12.0 mmol) was added to a stirring solution of 2-(phenylamino)acetonitrile **208** (660 mg, 5.0 mmol) in diethyl ether (5 mL) and the resulting suspension was stirred for 16 h in the absence of light. Dry hydrogen chloride (gas) was bubbled through solution for 15 min. The suspension was cooled in an ice bath for 15 min and filtered. The residue was dried under vacuum to give 3-phenylsydononimine hydrochloride **208** (927 mg, 4.70 mmol, 94%) as a pale pink solid, which was used without any further purification: **m.p.** 179-181, [Lit.[164] 180-181 °C]; **ν_{max}** (thin film)/cm^{-1} 2365, 1684, 1653, 1616, 1288, 1111; **^{1}H NMR** (400 MHz; d_6-DMSO) δ 10.10 (2H, s, N*H*.*H*Cl), 8.71 (1H, s, C*H*-*7*), 8.06 (2H, d, *J* 7.1 Hz, C*H*-3, 5) and 7.86-7.77 (3H, m, C*H*-1,2,6); **^{13}C NMR** (100 MHz; d_6-DMSO) δ 170.4 (quat., C-8), 134.5 (quat., C-4), 133.8 (quat.), 131.3 (*C*H × 2, C-3,5), 123.6 (*C*H × 2, C-2,6) and 103.2 (*C*H, C-7); ***m/z*** (ES$^+$) 162 ([M+H]$^+$, 100%); **HRMS** *m/z* (ES$^+$) calcd. for

$C_8H_8N_3O$ $[M+H]^+$ requires 162.0667, found 162.0663. The data were in agreement with the literature values.[94,164]

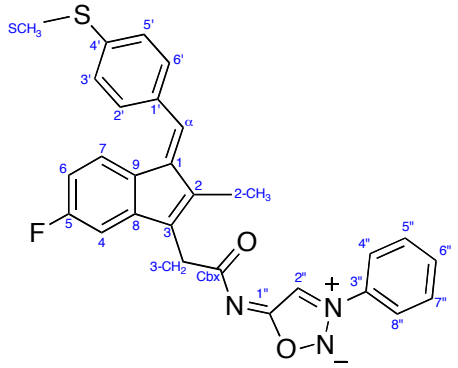
(*Z*)-(2-(5-Fluoro-2-methyl-1-(4-(methylsulfinyl)benzylidene)-1*H*-inden-3-yl)acetyl)(3-phenylsydnoniminyl)amide, 210



Sulindac **112** (50 mg, 0.14 mmol), EDCI.HCl (38 mg, 0.20 mmol) and DMAP (5 mg, 0.04 mmol) were added to a solution of 3-phenylsydnonimine **206** (28 mg, 0.14 mmol) in CH_2Cl_2:CH_3CN (4:1, 10 mL) and the resultant solution stirred at room temperature for 2 h. The solution was washed with distilled water (5 mL) and brine (5 mL), dried over $MgSO_4$, filtered and the solvent removed under reduced pressure to give crude product, which was purified by silica gel chromatography, eluting with dichloromethane and methanol (100:0 to 90:10), to afford *(Z)-(2-(5-fluoro-2-methyl-1-(4-(methylsulfinyl)benzylidene)-1H-inden-3-yl)acetyl)(3-phenylsydnoniminyl)amide* **210** (52 mg, 0.10 mmol, 74%) as a yellow solid: $\boldsymbol{R_f}$ 0.17 (95:5, CH_2Cl_2:MeOH, UV/cerium phosphomolybdate); **m.p.** 68-71 °C; $\boldsymbol{\nu_{max}}$ (thin film)/cm^{-1} 3052, 1719, 1634, 1601, 1558, 1468, 1414, 1360, 1248, 1163, 1046, 967. 920, 734; **^{1}H NMR** (400 MHz; $CDCl_3$) δ 8.48 (1H, s, C*H*-2″), 7.81 (2H, d, *J* 7.7 Hz, C*H*-3′,5′), 7.74-7.70 (3H, m, C*H*-4″,6″,8″), 7.68-7.65 (4H, m, C*H* × 2′,6′,5″,7″), 7.14-7.11 (2H, m, C*H*-7 and C*H*-α), 6.98 (1H, dd, *J* 8.9, 2.3 Hz, C*H*-4), 6.51 (1H, ddd, *J* 9.0, 8.9, 2.3 Hz, C*H*-6), 3.84 (2H, s, 3-CH_2), 2.82 (3H, s, SOCH_3) and 2.25 (3H, s, 2-CH_3); **^{13}C NMR** (100 MHz; $CDCl_3$) δ 178.2 (quat., C-1″), 173.0 (quat., carboxyl), 163.4 (quat., d, *J* 245.2 Hz, C-5), 147.6 (quat., d, *J* 9.0 Hz, C-8), 145.1 (quat., C-4′), 142.1 (quat., C-1), 140.1 (quat., C-1′), 137.8 (quat., C-2), 133.6 (quat., C-3″), 133.4 (quat., C-3), 130.6 (*C*H × 2, C-2′,6′), 130.3 (*C*H × 2, C-4″,8″) 130.2 (quat., C-9), 129.7 (*C*H, C-6″), 127.3 (*C*H, C-α), 123.7 (*C*H × 2, C-3′,5′), 123.3 (*C*H, d, *J* 8.9 Hz, C-7), 121.6 (*C*H × 2, C-5″,7″), 110.4 (*C*H, d, *J* 22.6 Hz, C-6), 106.5 (*C*H, d, *J* 24.3 Hz, C-4), 105.0 (*C*H, C-2″), 43.9 (*C*H_3, SOCH_3), 31.4 (*C*H_2, 3-CH_2) and 10.6 (*C*H_3, 2-CH_3); **^{19}F {^{1}H}** (376 MHz; $CDCl_3$) -113.2; ***m/z*** (ES^+) 500 [M+H], 522 ($[M+H]^+$, 100%); **HRMS** *m/z* (ES^+) calcd. for $C_{28}H_{23}FN3O_3S$ $[M+H]^+$ requires 500.1444, found 500.1446, $C_{28}H_{22}FN_3NaO_3S$ $[M+Na]^+$ requires 522.1264,

found 522.1263; **CHN** Anal. calcd. for $C_{28}H_{22}FN_3O_3S$: C, 67.32; H, 4.44; N, 8.41. Found C, 67.30; H, 4.46; N, 8.35.

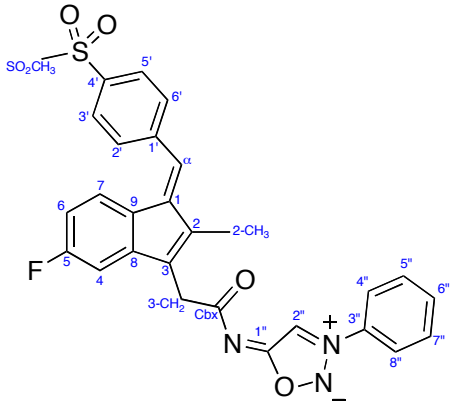
(*Z*)-(2-(5-Fluoro-2-methyl-1-(4-(methylthio)benzylidene)-1*H*-inden-3-yl)acetyl)(3-phenylsydnoniminyl)amide, 211



Sulindac sulfide **115** (50 mg, 0.15 mmol), EDCI.HCl (38 mg, 0.20 mmol) and DMAP (5 mg, 0.04 mmol) were added to a solution of 3-phenylsydnonimine **206** (30 mg, 0.15 mmol) in CH_2Cl_2:CH_3CN (4:1, 10 mL) and the resultant solution stirred at room temperature for 2 h. The solution was washed with distilled water (5 mL) and brine (5 mL), dried over $MgSO_4$, filtered and the solvent removed under reduced pressure to give crude product, which was purified by silica gel chromatography, eluting with dichloromethane and methanol (100:0 to 90:10), to afford *(Z)-(2-(5-fluoro-2-methyl-1-(4-(methylthio)benzylidene)-1H-inden-3-yl)acetyl)-(3-phenylsydnoniminyl)amide* **211** (46 mg, 0.10 mmol, 70%) as a yellow solid: $\boldsymbol{R_f}$ 0.51 (95:5, CH_2Cl_2:MeOH, UV/cerium phosphomolybdate); **m.p.** 54-56 °C; $\boldsymbol{\nu_{max}}$ (thin film)/cm^{-1} 1630, 1600, 1552, 1467, 1360, 1319, 1163, 1092, 967; **^{1}H NMR** (400 MHz; $CDCl_3$) δ 8.50 (1H, s, C*H*-2″), 7.88 (2H, d, *J* 7.8 Hz, C*H*-4″,8″), 7.78 (1H, tt, *J* 7.4, 2.1 Hz, C*H*-6), 7.73-7.67 (2H, m, C*H*-5″,7″), 7.43 (2H, d, *J* 8.3 Hz, C*H*-3′,5′), 7.34 (1H, dd, *J* 8.4, 5.0 Hz, C*H*-7), 7.28 (2H, d, *J* 7.8 Hz, C*H*-2′,6′), 7.12 (1H, s, C*H*-α), 6.97 (1H, dd, *J* 9.1, 2.4 Hz, C*H*-4), 6.53 (1H, ddd, *J* 9.0, 9.0, 2.4 Hz, C*H*-6), 4.03 (2H, s, 3-CH_2), 2.55 (3H, s, SCH_3) and 2.24 (3H, s, 2-CH_3); **^{13}C NMR** (100 MHz; $CDCl_3$) δ 178.4 (quat., C-1″), 173.2 (quat., carboxyl), 163.4 (quat., d, *J* 245.6 Hz, C-5), 146.9 (quat., d, *J* 9.1 Hz, C-8), 140.4 (quat., C-1), 139.3 (quat., C-2), 139.2 (quat., C-1′), 137.9 (quat., C-4′), 134.5 (*C*H, C-6″), 133.3 (quat., C-3″), 132.2 (quat., C-3), 131.2 (*C*H × 2, C-4″,8″), 130.2 (*C*H × 2, C-2′,6′), 130.1 (quat., C-9), 129.7 (*C*H, C-α), 126.2 (*C*H × 2, C-3′,5′), 123.8 (*C*H, d, *J* 8.6 Hz, C-7), 122.2 (*C*H × 2, C-5″,7″), 110.7 (*C*H, d, *J* 22.6 Hz, C-6), 106.4 (*C*H, d, *J* 23.8 Hz, C-4), 106.7 (*C*H, C-2″), 35.2 (*C*H_2, 3-CH_2), 15.7 (*C*H_3, S*C*H_3) and 11.1 (*C*H_3, 2-CH_3); **^{19}F {^{1}H} NMR** (376 MHz; $CDCl_3$) -114.2; ***m/z*** (ES^+) 484 ($[M+H]^+$, 100%); **HRMS** *m/z* (ES^+) calcd. for $C_{29}H_{22}FN_3NaO_2S$ $[M+Na]^+$ requires 506.1314, found 506.1310;

CHN Anal. calcd. for $C_{29}H_{22}FN_3O_2S$: C, 69.55; H, 4.59; N, 8.69. Found C, 69.56; H, 4.59; N, 8.72.

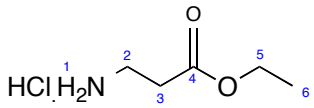
(*Z*)-(2-(5-Fluoro-2-methyl-1-(4-(methylsulfonyl)benzylidene)-1*H*-inden-3-yl)acetyl)(3-phenylsydnoniminyl)amide, 212



Sulindac sulfone **116** (50 mg, 0.13 mmol), EDCI.HCl (38 mg, 0.20 mmol) and DMAP (5 mg, 0.04 mmol) were added to a solution of 3-phenylsydnonimine **206** (28 mg, 0.14 mmol) in CH_2Cl_2:CH_3CN (4:1, 10 mL) and the resultant solution stirred at room temperature for 2 h. The solution was washed with distilled water (5 mL) and brine (5 mL), dried over anhydrous $MgSO_4$, filtered and the solvent removed under reduced pressure to give crude product which was purified by silica gel chromatography, eluting with dichloromethane and methanol (100:0 to 90:10), to afford *(Z)-(2-(5-fluoro-2-methyl-1-(4-(methylsulfonyl)benzylidene)-1H-inden-3-yl)-acetyl)(3-phenylsydnoniminyl)amide* **212** (64 mg, 0.13 mmol, 85%) as a yellow solid: $\boldsymbol{R_f}$ 0.52 (95:5, CH_2Cl_2:MeOH, UV/cerium phosphomolybdate); **m.p.** 62-65 °C; $\boldsymbol{\nu_{max}}$ (thin film)/cm^{-1} 3064, 1632, 1601, 1553, 1469, 1360, 1310, 1149, 964; **^{1}H NMR** (400 MHz; $CDCl_3$) δ 8.46 (1H, s, C*H*-2″), 7.97 (2H, d, *J* 8.4 Hz, C*H* × 3′,5′), 7.80-7.77 (2H, m, C*H*-4″,8″), 7.74-7.60 (5H, m, C*H*-2′,6′,5″,6″,7″), 7.07 (1H, s, C*H*-α), 7.05 (1H, dd, *J* 8.3, 5.0 Hz, C*H*-7) 6.96 (1H, dd, *J* 9.1, 2.4 Hz, C*H*-4), 6.49 (1H, ddd, *J* 9.1, 9.0, 2.4 Hz, C*H*-6), 3.82 (2H, s, 3-CH_2), 3.12 (3H, s, SO_2CH_3) and 2.22 (3H, s, 2-CH_3); **^{13}C NMR** (100 MHz; $CDCl_3$) δ 178.6 (quat., C-1″), 173.4 (quat., carboxyl), 162.9 (quat., d, *J* 246.1 Hz, C-5), 147.9 (quat., d, *J* 9.0 Hz, C-8), 143.0 (quat., C-4′), 143.1 (quat., C-1′), 139.8 (quat., C-1), 137.7 (quat., C-2), 135.2 (*C*H, C-6″), 133.8 (quat., C-3″), 133.6 (quat., C-3), 130.9 (*C*H × 2, C-2′,6′), 130.4 (*C*H × 2, C-4″,8″), 129.7 (quat., d, *J* 2.8 Hz, C-9), 127.7 (*C*H × 2, C-3′,5′), 126.5 (*C*H, C-α), 123.6 (*C*H, d, *J* 9.0 Hz, C-7), 121.7 (*C*H × 2, C-5″,7″), 110.7 (*C*H, d, *J* 22.8 Hz, C-6), 106.9 (*C*H, d, *J* 23.7 Hz, C-4), 105.1 (*C*H, C-2″), 44.7 (*C*H_3, SO_2CH_3), 37.1 (*C*H_2, 3-CH_2) and 10.8 (*C*H_3, 2-CH_3); **^{19}F {^{1}H} NMR** (376 MHz; $CDCl_3$) -113.2; ***m/z*** (ES^+) 516 ($[M+H]^+$, 100%); **HRMS** *m/z* (ES^+) calcd. for $C_{29}H_{22}FN_3NaO_4S$

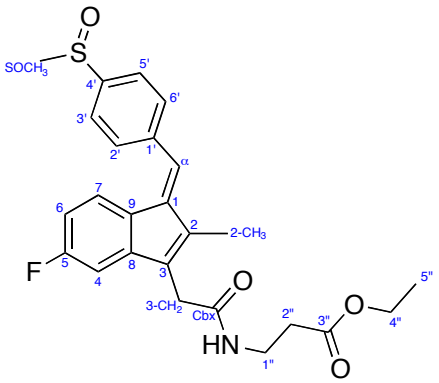
$[M+Na]^+$ requires 538.1213, found 538.1219; **CHN** Anal. calcd. for $C_{29}H_{22}FN_3O_4S$: C, 65.23; H, 4.30; N, 8.15. Found C, 65.22; H, 4.34; N, 8.17.

β-Alanine ethyl ester hydrochloride, 214[329]



Thionyl chloride (21.4 g. 13 mL, 0.18 mol) was added dropwise to a solution of β-alanine **213** (8.0 g, 0.09 mmol) in ethanol (200 mL) cooled in an ice bath. Following addition the solution was warmed to room temperature and stirred overnight. The solvent was removed under reduced pressure and the resulting solid was triturated with diethyl ether to provide β-alanine ethyl ester hydrochloride **214** (13.8 g, 0.09 mmol, quant) as a white solid, which was used without any further purification: **m.p.** 65-67 °C, [Lit.[401] 65-67 °C]; **^{1}H NMR** (400 MHz; d_6-DMSO) δ 8.09 (3H, s, NH_2.HCl-1), 4.06 (2H, q, ^3J 7.0, CH_2-5), 3.02 (2H, t, ^3J 6.8, CH_2-2), 2.68 (2H, t, ^3J 6.8, CH_2-3) and 1.17 (3H, t, ^3J 7.0, CH_3-6); **^{1}H NMR** (400 MHz; d_6-DMSO) δ 170.9 (quat., C-4), 61.1 (CH_2, C-5), 35.1 (CH_2, C-3), 31.7 (CH_2, C-2) and 14.4 (CH_3, C-6); ***m/z*** (ES^+) 118 ($[M+H]^+$, 100%). The data were in agreement with the literature values.[329,401]

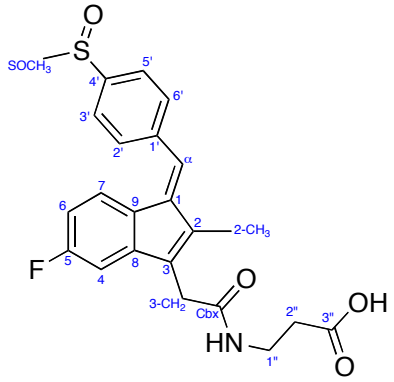
(*Z*)-Ethyl 3-(2-(5-fluoro-2-methyl-1-(4-(methylsulfinyl)benzylidene)-1*H*-inden-3-yl)acetamido)propanoate, 215



β-Alanine ethyl ester hydrochloride **214** (1.40 g, 9.00 mmol), dicyclohexylcarbodiimide (1.85 g, 9.00 mmol) and DMAP (20 mg, 0.20 mmol) were added to a solution of sulindac **112** (3.00 g, 8.42 mmol) and triethylamine (0.91 g, 1.26 mL, 9.00 mmol) in CH_2Cl_2 (30 mL) and the resultant solution was heated under reflux with stirring for 4 h. The solution was cooled, filtered and the residue triturated with ethyl acetate and cooled at 4 °C overnight. The solution was filtered and the filtrate washed with aq. HCl (2 N, 2 × 25 mL), The organic layer was separated and washed with distilled water (50 mL) and brine (50 mL), dried over $MgSO_4$, filtered and the

solvent removed under reduced pressure to yield a yellow oil, which was purified by silica gel chromatography, eluting with dichloromethane and methanol (99:1), to yield *(Z)-ethyl 3-(2-(5-fluoro-2-methyl-1-(4-(methylsulfinyl)benzylidene)-1H-inden-3-yl)acetamido)propanoate* **215** (3.49 g, 7.67 mmol, 91%) as a yellow crystalline solid: R_f 0.87 (90:10 CH_2Cl_2:MeOH); **m.p.** 59-60 °C; ν_{max} (thin film)/cm^{-1} 3301, 2929, 1732, 1647, 1602, 1551, 1467, 1370, 1196, 1171, 1036, 815; **^{1}H NMR** (400 MHz; $CDCl_3$) δ 7.74 (2H, d, ^3J 8.2, C*H*-3′,5′), 7.68 (2H, d, *J* 8.2, C*H*-2′,6′), 7.19 (1H, s, C*H*-α), 7.17 (1H, dd, *J* 8.5, 5.5, Hz, C*H*-7), 6.82 (1H, dd, *J* 8.8, 2.4 Hz, C*H*-4), 6.58 (1H, ddd, *J* 9.0, 8.8, 2.4 Hz, C*H*-6), 6.24 (1H, t, *J* 5.9 Hz, N*H*), 4.03 (2H, q, *J* 7.1 Hz, CH_2-4″), 3.50 (2H, s, 2-CH_2), 3.48 (2H, q, *J* 6.2 Hz, CH_2-1″), 2.82 (2H, s, SOCH_3), 2.50 (2H, t, *J* 6.2 Hz, CH_2-2″), 2.20 (3H, s, 2-CH_3) and 1.18 (3H, t, *J* 7.1 Hz, CH_3-5″); **^{13}C NMR** (100 MHz; $CDCl_3$) δ 172.4 (quat., C-3″), 169.4 (quat., carboxyl), 163.5 (quat., d, *J* 247.0 Hz, C-5), 146.6 (quat., d, *J* 8.5, C-8), 145.6 (quat., C-4′), 141.7 (quat., C-1), 139.7 (quat., C-1′), 138.8 (quat., C-2), 132.7 (quat., C-3), 130.4 (*C*H × 2, C-2′,6′), 129.7 (quat., C-9), 128.7 (*C*H, C-α), 124.0 (*C*H × 2, C-3′,5′), 123.8 (*C*H, d, *J* 9.4, C-7), 111.0 (*C*H, d, *J* 22.7 Hz, C-6), 106.1 (*C*H, d, *J* 23.9 Hz, C-4), 60.8 (*C*H_2, C-4″), 44.0 (*C*H_3, SO*C*H_3), 35.3 (*C*H_2, C-1″), 34.0 (*C*H_2, 3-*C*H_2), 33.8 (*C*H_2, C-2″), 14.2 (*C*H_3, C-5″) and 10.7 (*C*H_3, 2-*C*H_3); **^{19}F {^{1}H}** (376 MHz; $CDCl_3$) -112.4; ***m/z*** (ES$^+$) 478 ([M+Na]$^+$, 100%); **HRMS** *m/z* (ES$^+$) calcd. for $C_{25}H_{26}FNNaO_4S$ [M+Na]$^+$ requires 478.1464, found 478.1460.

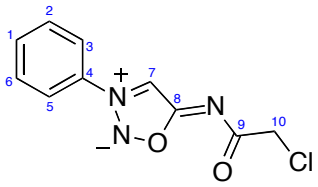
(*Z*)-3-(2-(5-Fluoro-2-methyl-1-(4-(methylsulfinyl)benzylidene)-1*H*-inden-3-yl)acetamido)propanoic acid, 216



Lithium hydroxide (178 mg, 7.43 mmol) was added in a single portion to a solution of ethyl ester **215** (564 mg, 1.24 mmol) in THF:H_2O (4:1, 20 mL) cooled in an ice bath. The resultant solution was stirred for one hour at 0 °C then stirred overnight at room temperature. The solution was cooled in an ice bath and aq. HCl (2 N, 10 mL) was added followed by extraction with dichloromethane (2 × 20 mL). The organic extracts were combined and washed with brine (25 mL), dried over $MgSO_4$, filtered and the solvent removed under reduced pressure to yield *(Z)-3-(2-(5-fluoro-2-methyl-1-(4-(methylsulfinyl)benzylidene)-1H-inden-3-yl)acetamido)-*

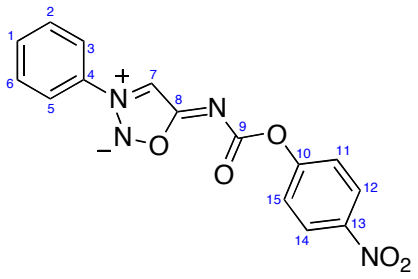
propanoic acid **216** (403 mg, 0.944 mmol, 76%) as a yellow crystalline solid: $\boldsymbol{R_f}$ 0.34 (90:10 CH_2Cl_2:MeOH); **m.p.** 63-65 °C; $\boldsymbol{\nu_{max}}$ (thin film)/cm^{-1} 3295, 3057, 2934, 1724, 1656, 1649, 1467, 1322,, 1196, 815; **^{1}H NMR** (400 MHz; $CDCl_3$) δ 7.77 (2H, d, *J* 8.6 Hz, C*H*-3′,5′), 7.72 (2H, d, *J* 8.2 Hz, C*H*-2′,6′), 7.18 (1H, s, C*H*-α), 7.16 (1H, dd, *J* 8.6, 5.2 Hz, C*H*-7), 6.84 (1H, dd, *J* 8.8, 2.3 Hz, C*H*-4), 6.56 (1H, ddd, *J* 9.0, 8.8, 2.3 Hz, C*H*-6), 6.33 (1H, t, *J* 6.1 Hz, N*H*), 3.52 (2H, s, 3-CH_2), 3.48 (2H, q, *J* 6.1 Hz, CH_2-1″), 2.83 (2H, s, SOCH_3), 2.54 (2H, t, ^{3}*J* 6.2, CH_2-2″) and 2.20 (3H, s, 2-CH3); **^{13}C NMR** (100 MHz; $CDCl_3$) δ 175.3 (quat., C-3″), 169.4 (quat., carboxyl), 163.4 (quat., d, *J* 245.3 Hz, C-5), 146.3 (quat., d, *J* 9.0, C-8), 145.2 (quat., C-4′), 141.5 (quat., C-1), 139.6 (quat., C-1′), 138.8 (quat., C-2), 132.7 (quat., C-3), 130.3 (*C*H × 2, C-2′,6′), 129.5 (quat., C-9), 128.6 (*C*H, C-α), 123.9 (*C*H × 2, C-3′,5′), 123.8 (*C*H, d, *J* 9.7, C-7), 111.1 (*C*H, d, *J* 22.7, C-6), 106.0 (*C*H, d, *J* 24.4, C-4), 43.6 (*C*H_3, SOCH_3), 35.0 (*C*H_2, C-1″), 33.6 (*C*H_2, C-2″), 33.2 (*C*H_2, 3-CH_2) and 10.5 (*C*H_3, 2-CH_3); **^{19}F NMR {^{1}H}** (376 MHz; $CDCl_3$) -112.8; ***m/z*** (ES^+) 450 ($[M+Na]^+$, 100%); ***m/z*** (ES^-) 426 ($[M-H]^-$, 100%); **HRMS** *m/z* (ES^+) calcd. for $C_{23}H_{22}FNNaO_4S$ $[M+Na]^+$ requires 450.1151, found 450.1154.

N-(2-Chloroacetyl)-3-phenylsydnonimine, 217



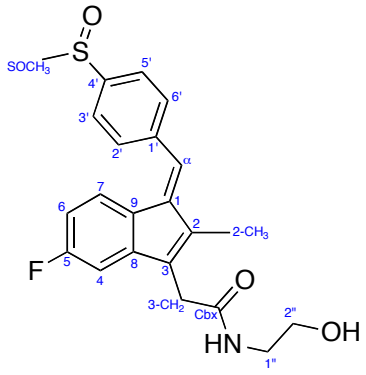
Triethylamine (236 mg, 325 µL, 2.33 mmol) was added to a solution of 3-phenylsydononimine hydrochloride **206** (200 mg, 1.02 mmol) and 2-bromoacetyl chloride (191 mg, 100 µL, 1.22 mmol) in CH_2Cl_2 (25 mL) at -20 °C. The solution was allowed to warm to room temperature overnight with continued stirring. The solution was partitioned between dichloromethane (100 mL) and distilled water (100 mL). The organic layer was separated and washed with distilled water (50 mL) and brine (50 mL), dried over Na_2SO_4, filtered and the solvent removed *in vacuo* to yield dark brown solid, which was purified by silica gel chromatography, eluting with dichloromethane and acetone (100:0 to 95:5), to provide *N*-(2-*chloroacetyl)-3-phenylsydnonimine* **217** (160 mg, 0.78 mmol, 66%) as a fawn solid: $\boldsymbol{R_f}$ 0.61 (90:10, CH_2Cl_2:acetone); **m.p.** 99-100 °C; $\boldsymbol{\nu_{max}}$ (thin film)/cm^{-1} 3121, 1647, 1556, 1468, 1400, 1362, 1337, 1239, 1176, 974, 939, 821; **^{1}H NMR** (300 MHz; $CDCl_3$) δ 8.51 (1H, s, C*H*-7), 7.84 (2H, d, *J* 7.8 Hz, C*H*-3,5), 7.79-7.67 (3H, m, C*H*-1,2,6) and 4.29 (2H, s, CH_2-10); **^{13}C NMR** (100 MHz; $CDCl_3$) δ 175.0 (quat., C-8), 174.0 (quat., C-9), 148.1 (quat., C-4), 133.9 (*C*H, C-1), 131.0 (*C*H × 2), 121.9 (*C*H × 2), 105.2 (*C*H, C-7) and 46.8 (*C*H_2, C-10); ***m/z*** (ES^+) 260 ($[M+Na]^+$, 100%); **HRMS** *m/z* (ES^+) calcd. for $C_{10}H_9N_3O_2Cl$ $[M+H]^+$ requires 238.0385, found 238.0383.

N-((4-Nitrophenoxy)carbonyl)-3-phenylsydnonimine, 218



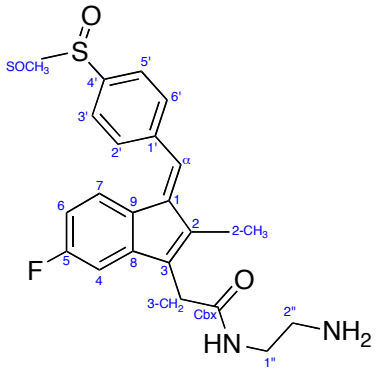
Triethylamine (1.18 g, 1.65 mL, 11.7 mmol) was added dropwise to a stirring solution of 3-phenylsydononimine hydrochloride **206** (1.00 g, 5.07 mmol) and *p*-nitrophenyl chloroformate (1.53 g, 7.61 mmol) in dichloromethane (20 mL) at -20 °C. The solution was allowed to warm to room temperature overnight with continued stirring. The solution was partitioned between dichloromethane (100 mL) and distilled water (100 mL). The organic layer was separated and washed with distilled water (50 mL) and brine (50 mL), dried over Na_2SO_4, filtered and the solvent removed *in vacuo* to give a pale yellow solid which was purified by silica gel chromatography, eluting with dichloromethane and acetone (100:0 to 95:5), to provide *N*-((*4-nitrophenoxy)carbonyl)-3-phenylsydnonimine* **218** (1.04 g, 3.19 mmol, 64%) as pale yellow needles: $\boldsymbol{R_f}$ 0.69 (90:10, CH_2Cl_2:acetone, $KMnO_4$); **m.p.** 198-200 °C (CH_3CN); $\boldsymbol{\nu_{max}}$ (thin film)/cm^{-1} 1700, 1671, 1587, 1576, 1513, 1490, 1345, 1276, 1216, 1187, 976, 765; **^{1}H NMR** (300 MHz; $CDCl_3$) δ 8.27 (2H, d, *J* 9.0 Hz, C*H*-12,14), 8.20 (1H, s, C*H*-7), 7.84 (2H, d, *J* 8.4, C*H*-3,5), 7.79-7.76 (1H, m, C*H*-1), 7.73-7.20 (2H, m, C*H*-2,6) and 7.40 (2H, d, *J* 9.0 Hz, C*H*-11,15); **^{13}C NMR** (100 MHz; $CDCl_3$) δ 176.1 (quat., C-8), 159.3 (quat., C-9″), 157.4 (quat., C-10″), 146.9 (quat., C-4″), 144.9 (quat., C-13″), 133.9 (*C*H, C-1″), 131.1 (*C*H × 2) 125.3 (*C*H × 2), 122.6 (*C*H × 2), 121.8 (*C*H × 2) and 103.9 (*C*H, C-7); ***m/z*** (ES^+) 349 ($[M+Na]^+$, 100%); **HRMS** *m/z* (ES^+) calcd. for $C_{15}H_{10}N_4NaO_5$ $[M+Na]^+$ requires 349.0549, found 349.0555.

(*Z*)-2-(5-Fluoro-2-methyl-1-(4-(methylsulfinyl)benzylidene)-1*H*-inden-3-yl)-*N*-(2-hydroxyethyl)acetamide, 221



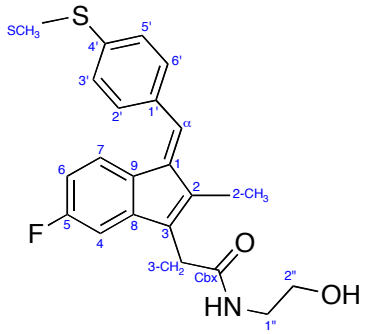
Sulindac **112** (1.00 g, 2.81 mmol) was added to a stirring solution of carbonyl diimidazole (0.50 g, 3.08 mmol) in dichloromethane (50 mL) and the solution stirred for 6 h with noticeable evolution of gas (CO_2). The reaction mixture was concentrated to half its volume and added to a stirring solution of ethanolamine **219** (1.88 g, 1.86 mL, 30.8 mmol) in dichloromethane (100 mL) and the resultant solution stirred overnight. This solution was washed with aq. NaOH (2 N, 2 × 50 mL). The organic layer was separated, dried over $MgSO_4$, filtered and the solvent removed under reduced pressure to give *(Z)-2-(5-fluoro-2-methyl-1-(4-(methylsulfinyl)benzylidene)-1H-inden-3-yl)-N-(2-hydroxyethyl)acetamide* **221** (0.99 g, 2.47 mmol, 88%), as a yellow crystalline solid, which was used without any further purification: $\boldsymbol{R_f}$ 0.55 (90:10, CH_2Cl_2:MeOH, UV/cerium phosphomolybdate); **m.p.** 79-80 °C; $\boldsymbol{\nu_{max}}$ (thin film)/cm^{-1} 3305, 3063, 1652, 1602, 1548, 1467, 1417, 1266, 1169, 1034, 815; **^{1}H NMR** (300 MHz; $CDCl_3$) δ 7.75 (2H, d, *J* 8.4 Hz, C*H*-3′,5′), 7.68 (2H, d, *J* 8.4 Hz, C*H*-2′,6′), 7.20 (1H, s, C*H*-α), 7.19 (1H, dd, *J* 8.5, 5.2 Hz, C*H*-7), 6.87 (1H, dd, *J* 8.7, 2.5 Hz, C*H*-4), 6.61 (1H, td, *J* 8.9, 8.8, 2.4 Hz, C*H*-6), 6.05 (1H, t, *br*, *J* 5.8 Hz, N*H*), 3.70 (2H, t, *J* 5.1 Hz, CH_2-2″), 3.56 (2H, s, 3-CH_2), 3.41 (2H, q, *J* 5.4 Hz, CH_2-1″), 2.82 (3H, s, SOCH_3), 2.40 (1H, s, *br*, O*H*) and 2.23 (3H, s, 2-CH_3); **^{13}C NMR** (100 MHz; $CDCl_3$) δ 170.6 (quat., carboxyl), 163.4 (quat., d, *J* 247.0 Hz, C-5), 146.0 (quat., d, *J* 9.0 Hz, C-8), 145.3 (quat., C-4′), 141.1 (quat., C-1), 139.7 (quat., C-1′), 139.1 (quat., C-2), 132.6 (quat., C-3), 130.5 (*C*H × 2, C-2′,6′), 130.2 (quat., C-9), 129.0 (*C*H, C-α), 124.3 (*C*H, d, *J* 9.0 Hz, C-7), 124.1 (*C*H × 2, C-3,5′), 111.7 (*C*H, d, *J* 22.1, C-6), 106.3 (*C*H, d, *J* 23.9 Hz, C-4), 62.7 (CH_2, C-2″), 44.2 (CH_3, $SOCH_3$), 42.9 (CH_2, C-2″), 33.9 (CH_2, 3-CH_2) and 10.9 (CH_3, 2-CH_3); **^{19}F {^{1}H} NMR** (376 MHz; $CDCl_3$) δ -112.7; ***m/z*** (ES^+) 422 ($[M+Na]^+$, 100%); **HRMS** *m/z* (ES^+) calcd. for $C_{22}H_{22}FNNaO_3S$ $[M+Na]^+$ requires 422.1202, found 422.1211.

(*Z*)-*N*-(2-Aminoethyl)-2-(5-fluoro-2-methyl-1-(4-(methylsulfinyl)benzylidene)-1*H*-inden-3-yl)acetamide, 222



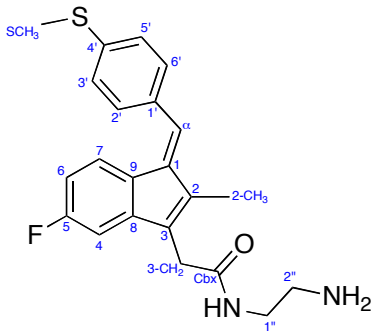
Sulindac **112** (1.00 g, 2.81 mmol) was added to a stirring solution of carbonyl diimidazole (0.50 g, 3.08 mmol) in dichloromethane (50 mL) and the solution stirred for 6 h with noticeable evolution of gas (CO_2). The reaction mixture was concentrated to half its volume and added to a stirring solution of ethylenediamine **220** (1.85 g, 2.07 mL, 30.8 mmol) in dichloromethane (100 mL) and the solution stirred overnight. The solution was washed with aq. NaOH (2 N, 2 × 50 mL). The organic layer was separated, dried over $MgSO_4$, filtered and the solvent removed under reduced pressure to give *(Z)-N-(2-aminoethyl)-2-(5-fluoro-2-methyl-1-(4-(methylsulfinyl)benzylidene)-1H-inden-3-yl)acetamide* **222** (0.90 g, 2.26 mmol, 81%), as a yellow crystalline solid, which was used without any further purification: $\boldsymbol{R_f}$ 0.59 (90:10, CH_2Cl_2:MeOH, UV/cerium phosphomolybdate); **m.p.** 78-79 °C; $\boldsymbol{\nu_{max}}$ (thin film)/cm^{-1} 3278, 3050, 1655, 1651, 1601, 1557, 1467, 1168, 1087, 1038, 956, 733; **^{1}H NMR** (300 MHz; $CDCl_3$) δ 7.74 (2H, d, *J* 8.5 Hz, C*H*-3′,5′), 7.68 (2H, d, *J* 8.5 Hz, C*H*-2′,6′), 7.19 (1H, s, C*H*-α), 7.18 (1H, dd, *J* 8.4, 5.1 Hz, C*H*-7), 6.88 (1H, dd, *J* 8.7, 2.3 Hz, C*H*-4), 6.59 (1H, ddd, *J* 8.9, 8.8, 2.5 Hz, C*H*-6), 6.09 (1H, s, *br*, N*H*), 3.54 (2H, s, 3-CH_2), 3.27 (2H, q, *J* 5.8 Hz, CH_2-1″), 2.83 (3H, s, SOCH_3), 2.76 (2H, t, *J* 6.1 Hz, CH_2-2″) and 2.22 (3H, s, 2-CH_3); **^{13}C NMR** (100 MHz; $CDCl_3$) δ 169.8 (quat., carboxyl), 163.8 (quat., d, *J* 246.5 Hz, C-5), 147.1 (quat., d, *J* 8.7 Hz, C-8), 145.3 (quat., C-4′), 141.5 (quat., C-1), 139.6 (quat., C-1′), 138.5 (quat., C-2), 132.8 (quat., d, *J* 2.2, C-3), 130.3 (*C*H × 2, C-2′,6′), 129.6 (quat., d, *J* 2.7 Hz, C-9), 128.4 (*C*H, C-α), 123.8 (*C*H × 2, C-3,5′), 123.6 (*C*H, d, *J* 9.3 Hz, C-7), 110.9 (*C*H, d, *J* 22.7 Hz, C-6), 106.0 (*C*H, d, *J* 23.9 Hz, C-4), 43.8 (*C*H_3, SOCH_3), 42.4 (*C*H_2, C-1″), 41.3 (*C*H_2, C-2″), 33.6 (*C*H_2, 3-CH_2) and 10.6 (*C*H_3, 2-CH_3); **^{19}F {^{1}H} NMR** (376 MHz; $CDCl_3$) δ -112.8; ***m/z*** (ES^+) 421 ($[M+Na]^+$, 100%); **HRMS** *m/z* (ES^+) calcd. for $C_{22}H_{23}FN_2NaO_2S$ $[M+Na]^+$ requires 421.1362, found 421.1360.

(*Z*)-2-(5-Fluoro-2-methyl-1-(4-(methylthio)benzylidene)-1*H*-inden-3-yl)-*N*-(2-hydroxyethyl)acetamide, 223



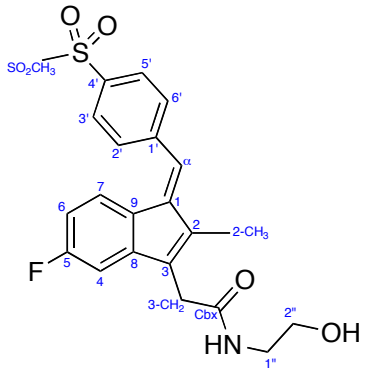
Sulindac sulfide **115** (0.35 g, 1.03 mmol) was added to a stirring solution of carbonyl diimidazole (0.180 g, 1.10 mmol) in dichloromethane (50 mL) and the solution stirred for 6 h with noticeable evolution of gas (CO_2). The reaction mixture was concentrated to half its volume and added to a stirring solution of ethanolamine **219** (1.02 g, 1.10 mL, 16.9 mmol) in dichloromethane (100 mL) and the solution stirred overnight. The solution was washed with aq. NaOH (2 N, 2 × 50 mL). The organic layer was separated, dried over $MgSO_4$, filtered and the solvent removed under reduced pressure to give a yellow residue. This residue was purified by silica gel chromatography, eluting with dichloromethane and methanol (100:0 to 85:15), to afford *(Z)-2-(5-fluoro-2-methyl-1-(4-(methylthio)benzylidene)-1H-inden-3-yl)-N-(2-hydroxyethyl)acetamide* **223** (0.34 g, 0.88 mmol, 85%) as a yellow solid: $\boldsymbol{R_f}$ 0.65 (90:10, CH_2Cl_2:MeOH, UV/cerium phosphomolybdate); **m.p.** 67-69 °C; $\boldsymbol{\nu_{max}}$ (thin film)/cm^{-1} 3277, 1652, 1601, 1543, 1307, 1145, 1088, 1066, 960; **^{1}H NMR** (300 MHz; d_6-DMSO) δ 8.13 (1H, t, *J* 5.5 Hz, N*H*), 7.49 (2H, d, *J* 8.3 Hz, C*H*-3′,5′), 7.36-7.30 (3H, m, C*H*-2′,6′, 7), 7.26 (1H, s, C*H*-α), 7.12 (1H, dd, *J* 9.5, 2.5 Hz, C*H*-4), 6.71 (1H, ddd, *J* 9.0, 8.5, 2.5 Hz, C*H*-6), 4.71 (1H, t, *J* 5.1 Hz, O*H*), 3.44-3.88 (4H, m, 3-CH_2, CH_2-2″), 3.13 (2H, q, *J* 6.0 Hz, CH_2-1″), 2.53 (3H, s, SCH_3) and 2.16 (3H, s, 2-CH_3); **^{13}C NMR** (d_6-DMSO) δ 168.9 (quat., carboxyl), 162.4 (quat., d, *J* 243.4 Hz, C-5), 147.1 (quat., d, *J* 9.1 Hz, C-8), 139.2 (quat., C-1), 138.9 (quat., C-2), 137.8 (quat., C-4′), 132.9 (quat., d, *J* 2.3 Hz, C-9), 132.7 (quat., C-3), 130.1 (quat., C-1′), 129.8 (*C*H × 2, C-2′,6′), 129.6 (*C*H, C-α), 125.5 (*C*H × 2, C-3,5′), 122.9 (*C*H, d, *J* 9.1 Hz, C-7), 110.1 (*C*H, d, *J* 22.8 Hz, C-6), 106.0 (*C*H, d, *J* 23.7 Hz, C-4), 59.8 (*C*H_2-4″, C-2″), 41.6 (*C*H_2, C-1″), 32.7 (*C*H_2, 3-CH_2), 14.3 (*C*H_3, S*C*H_3) and 10.4 (*C*H_3, 2-CH_3); **^{19}F {^{1}H} NMR** (376 MHz; $CDCl_3$) δ -114.6; ***m/z*** (ES^+) 406 ($[M+Na]^+$, 100%); **HRMS** *m/z* (ES^+) calcd. for $C_{22}H_{22}FNNaO_2S$ $[M+Na]^+$ requires 406.1253, found 406.1255.

(*Z*)-*N*-(2-Aminoethyl)-2-(5-fluoro-2-methyl-1-(4-(methylthio)benzylidene)-1*H*-inden-3-yl)acetamide, 224



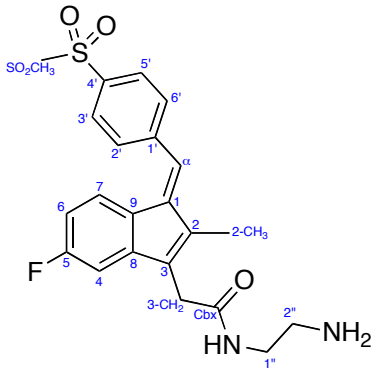
Sulindac sulfide **115** (0.350 g, 1.03 mmol) was added to a stirring solution of carbonyl diimidazole (0.180 g, 1.10 mmol) in dichloromethane (50 mL) and the solution stirred for 6 h with noticeable evolution of gas (CO_2). The reaction mixture was concentrated to half its volume and added to a stirring solution of ethylenediamine **220** (1.14 g, 1.10 mL, 16.9 mmol) in dichloromethane (100 mL) and the solution stirred overnight. The solution was washed with aq. NaOH (2 N, 2 × 50 mL). The organic layer was separated, dried over $MgSO_4$, filtered and the solvent removed under reduced pressure to give a yellow residue. This residue was purified by silica gel chromatography, eluting with dichloromethane and methanol (100:0 to 85:15), to afford *(Z)-N-(2-aminoethyl)-2-(5-fluoro-2-methyl-1-(4-(methylthio)benzylidene)-1H-inden-3-yl)acetamide* **224** (0.35 g, 0.92 mmol, 89%) as a yellow solid: $\boldsymbol{R_f}$ 0.58 (90:10, CH_2Cl_2:MeOH, UV/cerium phosphomolybdate); **m.p.** 67-69 °C; $\boldsymbol{\nu_{max}}$ (thin film)/cm^{-1} 3419, 2088, 1646, 1491, 1465, 1320, 1262, 1170; **^{1}H NMR** (300 MHz; d_6-DMSO) δ 8.46 (1H, t, *J* 5.4, N*H*), 7.48 (2H, d, *J* 8.3 Hz, C*H*-3′,5′), 7.36-7.30 (3H, m, C*H*- 2′,6′,7), 7.27 (1H, s, C*H*-α), 7.13 (1H, dd, *J* 9.4, 2.4 Hz, C*H*-4), 6.72 (1H, ddd, *J* 9.0, 8.4, 2.4 Hz, C*H*-6), 3.46 (2H, s, 3-CH_2), 3.25 (2H, q, *J* 6.2 Hz, CH_2-1″), 2.79 (2H, t, *J* 6.2 Hz, CH_2-2″), 2.53 (3H, s, SCH_3) and 2.17 (3H, s, 2-CH_3); **^{13}C NMR** (d_6-DMSO) δ 169.4 (quat., carboxyl), 162.4 (quat., d, *J* 242.5 Hz, C-5), 147.1 (quat., d, *J* 9.1 Hz, C-8), 139.2 (quat., C-1), 139.0 (quat., C-2), 138.0 (quat., C-4′), 132.6 (quat., C-9), 132.3 (quat., C-3), 130.2 (quat., C-1′), 129.9 (*C*H × 2, C-2′,6′), 129.6 (*C*H, C-α), 125.5 (*C*H × 2, C-3,5′), 122.9 (*C*H, d, *J* 9.3 Hz, C-7), 110.1 (*C*H, d, *J* 22.7 Hz, C-6), 106.1 (*C*H, d, *J* 23.7 Hz, C-4), 38.6 (*C*H_2, C-1″), 38.0 (*C*H_2, C-2″), 32.7 (*C*H_2, 3-CH_2), 14.3 (*C*H_3, SCH_3) and 10.5 (*C*H_3, 2-CH_3); **^{19}F {^{1}H} NMR** (376 MHz; $CDCl_3$) δ -114.5; ***m/z*** (ES^+) 405 ([M+Na]$^+$, 100%); **HRMS** *m/z* (ES^+) calcd. for $C_{22}H_{23}FN_2NaOS$ [M+Na]$^+$ requires 405.1413, found 405.1409.

(*Z*)-2-(5-Fluoro-2-methyl-1-(4-(methylsulfonyl)benzylidene)-1*H*-inden-3-yl)-*N*-(2-hydroxyethyl)acetamide, 225



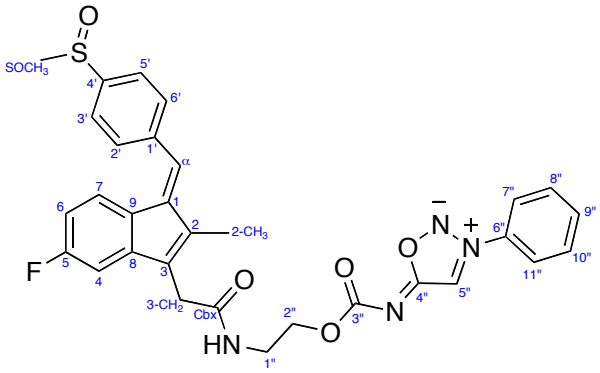
Sulindac sulfone **116** (0.35 g, 0.94 mmol) was added to a stirring solution of carbonyl diimidazole (0.18 g, 1.10 mmol) in dichloromethane (50 mL) and the solution stirred for 6 h with noticeable evolution of gas (CO_2). The reaction mixture was concentrated to half its volume and added to a stirring solution of ethanolamine **219** (1.02 g, 1.10 mL, 16.9 mmol) in dichloromethane (100 mL) and the solution stirred overnight. The solution was washed with aq. NaOH (2 N, 2 × 50 mL). The organic layer was separated, dried over $MgSO_4$, filtered and the solvent removed under reduced pressure to give a yellow residue. This residue was purified by silica gel chromatography, eluting with dichloromethane, methanol and triethylamine (100:0 to 95:5), to afford *(Z)-2-(5-fluoro-2-methyl-1-(4-(methylsulfonyl)benzylidene)-1H-inden-3-yl)-N-(2-hydroxyethyl)acetamide* **225** (0.35 g, 0.85 mmol, 90%) as a yellow solid: $\boldsymbol{R_f}$ 0.60 (90:10, CH_2Cl_2:MeOH, UV/cerium phosphomolybdate); **m.p.** 120-122 °C; $\boldsymbol{\nu_{max}}$ (thin film)/cm^{-1} 3295, 1644, 1600, 1548, 1491, 1466, 1262, 1169, 1092; **^{1}H NMR** (300 MHz; d_6-DMSO) δ 8.16 (1H, t, *J* 5.6 Hz, N*H*), 8.03 (2H, d, *J* 8.4 Hz, C*H*-3′,5′), 7.85 (2H, d, *J* 8.4 Hz, C*H*-2′,6′), 7.35 (1H, s, C*H*-α), 7.14-7.09 (2H, m, C*H*-7, C*H*-4), 6.70 (1H, ddd, *J* 9.0, 8.9, 2.4 Hz, C*H*-6), 4.70 (1H, t, *J* 5.3, O*H*), 3.44-3.40 (4H, m, 3-CH_2 and CH_2-2″), 3.29 (3H, s, SO_2CH_3), 3.13 (2H, q, *J* 6.1 Hz, CH_2-1″) and 2.18 (3H, s, 2-CH_3); **^{13}C NMR** (d_6-DMSO) δ 168.7 (quat., carboxyl), 162.6 (quat., d, *J* 243.9 Hz, C-5), 147.4 (quat., d, *J* 9.6 Hz, C-8), 141.5 (quat., C-4′), 141.1 (quat., C-1′), 140.1 (quat., C-1), 137.6 (quat., C-2), 134.2 (quat., C-3), 130.0 (*C*H × 2, C-2′,6′), 129.3 (quat., d, *J* 2.3 Hz, C-9), 128.3 (*C*H, C-α), 127.2 (*C*H × 2, C-3,5′), 123.1 (*C*H, d, *J* 9.3 Hz, C-7), 110.3 (*C*H, d, *J* 22.7 Hz , C-6), 106.4 (*C*H, d, *J* 24.5 Hz, C-4), 59.8 (*C*H_2, C-2″), 43.4 (*C*H_3, SO_2CH_3), 41.6 (CH_2, C-1″), 32.7 (*C*H_2, 3-CH_2) and 10.3 (*C*H_3, 2-CH_3); **^{19}F {^{1}H} NMR** (376 MHz; $CDCl_3$) δ -113.8; ***m/z*** (ES^+) 438 ($[M+Na]^+$, 100%); **HRMS** *m/z* (ES^+) calcd. for $C_{22}H_{22}FNNaO_4S$ $[M+Na]^+$ requires 438.1151, found 438.1155.

(*Z*)-*N*-(2-Aminoethyl)-2-(5-fluoro-2-methyl-1-(4-(methylsulfonyl)benzylidene)-1*H*-inden-3-yl)acetamide, 226



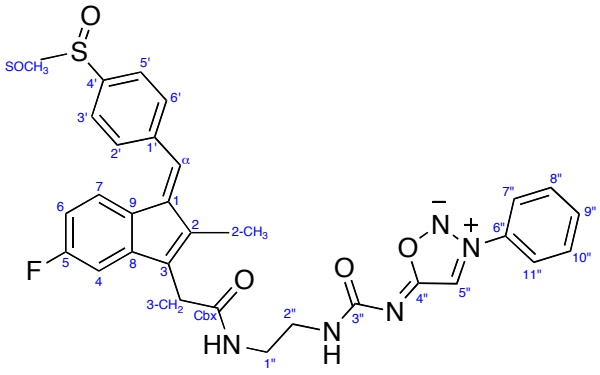
Sulindac sulfone **116** (0.35 g, 0.94 mmol) was added to a stirring solution of carbonyl diimidazole (0.18 g, 1.10 mmol) in dichloromethane (50 mL) and the solution stirred for 6 h with noticeable evolution of gas (CO_2). The reaction mixture was concentrated to half its volume and added to a stirring solution of ethylenediamine **220** (1.14 g, 1.10 mL, 16.9 mmol) in dichloromethane (100 mL) and the solution stirred overnight. The solution was washed with aq. NaOH (2 N, 2 × 50 mL). The organic layer was separated, dried over $MgSO_4$, filtered and the solvent removed under reduced pressure to give a yellow residue. This residue was purified by silica gel chromatography, eluting with dichloromethane, methanol and triethylamine (100:0:0 to 85:15:1), to afford *(Z)-N-(2-aminoethyl)-2-(5-fluoro-2-methyl-1-(4-(methylsulfonyl)benzylidene)-1H-inden-3-yl)acetamide* **226** (0.32 g, 0.78 mmol, 83%) as a yellow solid: $\boldsymbol{R_f}$ 0.43 (90:10, CH_2Cl_2:MeOH, UV/cerium phosphomolybdate); **m.p.** 128-130 °C; $\boldsymbol{\nu_{max}}$ (thin film)/cm^{-1} 3285, 1651, 1601, 1548, 1306, 1148, 1089, 1016, 960; **^{1}H NMR** (300 MHz; d_6-DMSO) δ 8.16 (1H, t, *J* 5.7, N*H*), 8.02 (2H, d, *J* 8.5 Hz, C*H*-3′,5′), 7.88 (2H, d, *J* 8.5 Hz, C*H*-2′,6′), 7.35 (1H, s, C*H*-α), 7.15-7.10 (2H, m, C*H*-4, 7), 6.70 (1H, ddd, *J* 9.0, 9.0, 2.4 Hz, C*H*-6), 3.44 (2H, s, 3-CH_2), 3.29 (3H, s, SO_2CH_3), 3.09 (2H, q, *J* 6.3 Hz, CH_2-1″), 2.62 (2H, t, *J* 6.3 Hz, CH_2-2″) and 2.18 (3H, s, 2-CH_3); **^{13}C NMR** (100 MHz, d_6-DMSO) δ 168.8 (quat., carboxyl), 162.8 (quat., d, *J* 243.6 Hz, C-5), 147.4 (quat., d, *J* 8.8 Hz, C-8), 141.5 (quat., C-4′), 141.1 (quat., C-1′), 140.1 (quat., C-1), 137.0 (quat., C-2), 134.2 (quat., C-3), 130.4 (*C*H × 2, C-2′,6′), 129.3 (quat., C-9), 128.4 (*C*H, C-α), 127.2 (*C*H × 2, C-3,5′), 123.1 (*C*H, d, *J* 9.2, C-7), 110.4 (*C*H, d, *J* 22.7 Hz, C-6), 106.3 (*C*H, d, *J* 23.0 Hz, C-4), 43.4 (*C*H_3, SO_2CH_3), 41.5 (*C*H_2, NHC-1″), 40.8 (*C*H_2NH_2, C-2″), 32.7 (*C*H_2, 3-CH_2) and 10.4 (*C*H_3, 2-CH_3); **^{19}F {^{1}H} NMR** (376 MHz; $CDCl_3$) δ -113.8; ***m/z*** (ES^+) 437 ([M+Na]$^+$, 100%); **HRMS** *m/z* (ES^+) calcd. for $C_{22}H_{23}FN_2NaO_3S$ [M+Na]$^+$ requires 437.1311, found 437.1310.

_N_-(((2-(2-((_Z_)-5-Fluoro-2-methyl-1-(4-(methylsulfinyl)benzylidene)-1H-inden-3-yl)acetamido)ethoxy)carbonyl)-3-phenylsydnonimine, 227



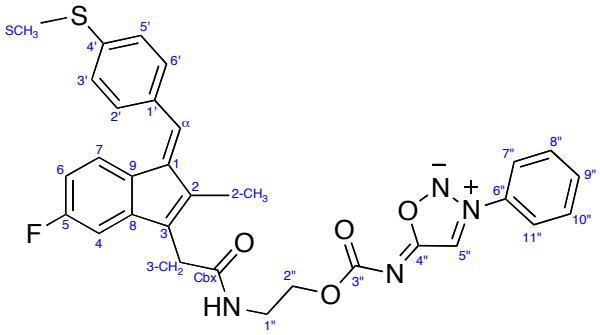
Alcohol **221** (100 mg, 0.25 mmol) and *N*-((4-nitrophenoxy)carbonyl)-3-phenylsydnonimine **218** (82 mg, 0.25) were dissolved in acetonitrile (10 mL) and heated under reflux for 24 h. The solvent was removed under reduced pressure and the residue purified by silica gel chromatography, eluting with dichloromethane and methanol (100: 0 to 90:10), to provide (*Z*)-*5*-(((*2*-(*2*-((*Z*)-*5*-*fluoro*-*2*-*methyl*-*1*-(*4*-(*methylsulfinyl)benzylidene)-1H-inden-3-yl)acetamido)ethoxy)carbonyl)-3-phenylsydnonimine* **227** (25 mg, 0.04 mmol, 17%) as a yellow solid: $\boldsymbol{R_f}$ 0.77 (90:10, CH_2Cl_2:MeOH, UV/cerium phosphomolybdate); **m.p.** (acetone) 89-91 °C; $\boldsymbol{\nu_{max}}$ (thin film)/cm^{-1} 3292, 3064, 1722, 1662, 1604, 1591, 1468, 1369, 1279, 1199, 1039, 969, 858, 815, 733; **^{1}H NMR** (400 MHz; $CDCl_3$) δ 8.08 (1H, s, C*H*-5″), 7.80 (2H, d, *J* 8.0 Hz, C*H*-3′,5′), 7.74-7.55 (7H, m, C*H*-2′,6′,7″,8″,9″,10″,11″), 7.16 (1H, s, C*H*-α), 7.10 (1H, dd, *J* 8.4, 5.1 Hz, C*H*-7), 6.84 (1H, dd, *J* 8.9, 2.4 Hz, C*H*-4), 6.47 (1H, ddd, *J* 9.0, 8.9, 2.4 Hz, C*H*-6), 6.46 (1H, t, *J* 5.6 Hz, N*H*), 4.21 (2H, t, *J* 5.2 Hz, CH_2-2″), 3.58 (2H, q, *J* 5.2 Hz, CH_2-1″), 3.52 (2H, s, 3-CH_2), 2.81 (3H, s, SOCH_3) and 2.21 (3H, s, 2-CH_3); **^{13}C NMR** (100 MHz; $CDCl_3$) δ 175.3 (quat., C-4″), 169.6 (quat., carboxyl), 163.6 (quat., d, *J* 246.6 Hz, C-5), 161.4 (quat., C-3″), 146.8 (quat., d, *J* 8.9 Hz, C-8), 145.7 (quat., C-4′), 141.9 (quat., C-1), 139.8 (quat., C-1′), 139.8 (C-2, quat.), 134.0 (quat., C-6″), 133.5 (*C*H, C-9″), 132.7 (quat., C-3), 130.9 (*C*H × 2, C-7″,11″), 130.6 (*C*H × 2, C-4′,6′), 130.5 (quat., C-9), 128.7 (*C*H, C-α), 124.1 (*C*H × 2, C-3′,5′), 123.9 (*C*H, d, *J* 8.9 Hz, C-7), 121.8 (*C*H × 2, C-8″,10″), 111.1 (*C*H, d, *J* 22.6 Hz, C-6), 106.3 (*C*H, d, *J* 24.1 Hz, C-4), 103.2 (*C*H, C-5″), 64.7 (*C*H_2, C-2″), 44.2 (*C*H_3, SOCH_3), 39.5 (*C*H_2, C-1″), 34.0 (*C*H_2, 3-CH_2) and 10.9 (*C*H_3, 2-CH_3); **^{19}F {^{1}H} NMR** (376 MHz; $CDCl_3$) δ -113.1; ***m/z*** (ES^+) 609 ([M+Na]$^+$, 100%); **HRMS** *m/z* (ES^+) calcd. for $C_{31}H_{27}FN_4NaO_5S$ [M+Na]$^+$ requires 609.1584, found 609.1591; **CHN** Anal. calcd. for $C_{31}H_{27}FN_4O_5S$: C, 63.47; H, 4.64; N, 9.55. Found C, 63.50; H, 4.70; N, 9.59.

_N_-(((2-(2-((_Z_)-5-Fluoro-2-methyl-1-(4-(methylsulfinyl)benzylidene)-1_H_-inden-3-yl)acetamido)ethoxy)carbamoyl)-3-phenylsydnonimine, 228



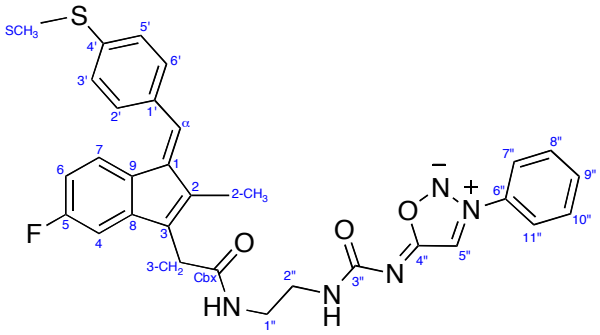
Amine **222** (100 mg, 0.25 mmol) and *N*-((4-nitrophenoxy)carbonyl)-3-phenylsydnonimine **218** (82 mg, 0.25) were dissolved in acetonitrile (10 mL) and heated under reflux for 24 h. The solvent was removed under reduced pressure and the residue purified by silica gel chromatography, eluting with dichloromethane and methanol (100: 0 to 90:10), to provide *(Z)-5-(((2-(2-((Z)-5-fluoro-2-methyl-1-(4-(methylsulfinyl)benzylidene)-1H-inden-3-yl)acetamido)ethoxy)-carbamoyl)-3-phenylsydnonimine* **228** (126 mg, 0.22 mmol, 86%) as a yellow solid: $\boldsymbol{R_f}$ 0.53 (90:10, CH_2Cl_2:MeOH, UV/cerium phosphomolybdate); **m.p.** 118-120 °C; $\boldsymbol{\nu_{max}}$ (thin film)/cm^{-1} 3428, 1728, 1632, 1602, 1526, 1468, 1368, 1295, 1136, 960; **^{1}H NMR** (400 MHz; $CDCl_3$) δ 8.02 (1H, s, C*H*-5″), 7.74-7.61 (9H, m, C*H*-2′,3′,5′,6′,7″,8″,9″, 10″,11″), 7.13 (1H, s, C*H*-α), 7.11 (1H, dd, *J* 8.3, 5.1 Hz, C*H*-7), 6.88 (1H, dd, *J* 8.8, 2.4 Hz, C*H*-4), 6.70 (1H, s, *br*, N*H*), 6.47 (1H, ddd, *J* 9.0, 8.8, 2.4 Hz, C*H*-6), 5.78 (1H, s, *br*, N*H*), 3.50 (2H, s, 3-CH_2), 3.42-3.36 (4H, m, CH_2-1″, 2″), 2.81 (3H, s, SOCH_3) and 2.21 (3H, s, 2-CH_3); **^{13}C NMR** (100 MHz; $CDCl_3$) 173.0 (quat., C-4″), 169.8 (quat., carboxyl), 163.6 (quat., d, *J* 248.0 Hz, C-5), 162.9 (quat., C-3″), 147.0 (quat., d, *J* 8.8 Hz, C-8), 145.8 (quat., C-4′), 141.9 (quat., C-1), 139.9 (quat., C-1′), 138.9 (C-2, quat.), 134.2 (quat., C-6″), 133.2 (*C*H, C-9″), 133.0 (quat., C-3), 130.7 (*C*H × 2, C-7″,11″), 130.5 (*C*H × 2, C-4′,6′) 129.9 (quat., C-9), 128.5 (*C*H, C-α), 124.1 (*C*H × 2, C-3′,5′), 123.8 (*C*H, d, *J* 8.8 Hz, C-7), 121.7 (*C*H × 2, C-8″,10″), 111.0 (*C*H, d, *J* 22.5 Hz, C-6), 106.3 (*C*H, d, *J* 23.2 Hz, C-4), 102.2 (*C*H, C-5″), 44.2 (*C*H_3, SOCH_3), 41.1 (*C*H_2, CH_2, C-2″), 40.6 (*C*H_2, C-1″), 34.1 (*C*H_2, 3-CH_2) and 10.9 (*C*H_3, 2-CH_3); **^{19}F {^{1}H} NMR** (376 MHz; $CDCl_3$) δ -113.2; ***m/z*** (ES^+) 608 ([M+Na]$^+$, 100%); **HRMS** *m/z* (ES^+) calcd. for $C_{31}H_{28}FN_5NaO_4S$ [M+Na]$^+$ requires 608.1744, found 608.1745; **CHN** Anal. calcd. for $C_{31}H_{28}FN_5O_4S$: C, 63.58; H, 4.82; N, 11.96. Found C, 63.66; H, 4.89; N, 12.00.

_N_-(((2-(2-((_Z_)-5-Fluoro-2-methyl-1-(4-(methylthio)benzylidene)-1_H_-inden-3-yl)acetamido)ethoxy)carbonyl)-3-phenylsydnonimine, 229



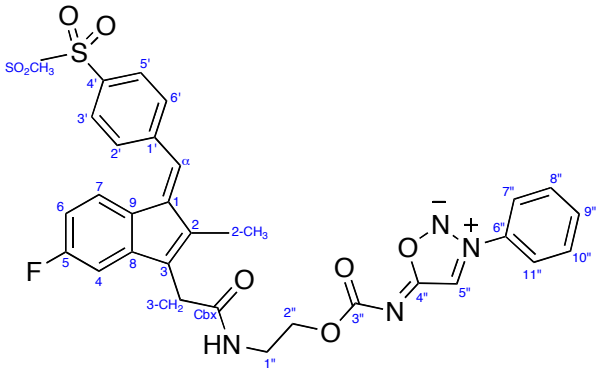
Alcohol **223** (100 mg, 0.26 mmol) and *N*-((4-nitrophenoxy)carbonyl)-3-phenylsydnonimine **218** (85 mg, 0.26) were dissolved in acetonitrile (10 mL) and heated under reflux for 60 h. The solvent was removed under reduced pressure and the residue purified by silica gel chromatography, eluting with dichloromethane and methanol (100:0 to 90:10), to provide *N*-(((*2*-(*2*-((*Z*)-*5-fluoro-2-methyl-1*-(*4*-(*methylthio)benzylidene)-1H-inden-3-yl)acetamido)ethoxy)-carbonyl)-3-phenylsydnonimine* **229** (71 mg, 0.118 mmol, 56%) as a yellow solid: $\boldsymbol{R_f}$ 0.78 (90:10, CH_2Cl_2:MeOH, UV/cerium phosphomolybdate); **m.p.** 81-83 °C; $\boldsymbol{\nu_{max}}$ (thin film)/cm^{-1} 1658, 1603, 1589, 1467, 1369, 1278, 1198, 1070, 970; **^{1}H NMR** (400 MHz; $CDCl_3$) δ 8.03 (1H, s, C*H*-5″), 7.80-7.62 (4H, m, C*H*-3′,5′,7″,11″), 7.47-7.42 (1H, m, C*H*-9″), 7.40 (1H, dd, *J* 8.4, 5.1 Hz, C*H*-7), 7.33-7.26 (4H, m, C*H*-2′,6′,8″,10″), 7.13 (1H, s, C*H*-α), 6.84 (1H, dd, *J* 9.0, 2.3 Hz, C*H*-4), 6.48 (1H, ddd, *J* 9.0, 9.0, 2.3 Hz, C*H*-6), 6.30 (1H, t, *J* 5.7 Hz, N*H*), 4.20 (2H, t, *J* 5.1 Hz, CH_2-2″), 3.57 (2H, q, *J* 5.4 Hz, CH_2-1″), 3.52 (2H, s, 3-CH_2), 2.55 (3H, s, SCH_3) and 2.19 (3H, s, 2-CH_3); **^{13}C NMR** (100 MHz; $CDCl_3$) δ 175.3 (quat., C-4″), 169.9 (quat., carboxyl), 163.4 (quat., d, *J* 246.6 Hz, C-5), 161.4 (quat., C-3″), 146.5 (quat., d, *J* 8.8 Hz, C-8), 140.2 (quat., C-1), 140.0 (C-2, quat.), 139.5 (quat., C-4′), 139.4 (quat., C-1′), 134.0 (quat., C-6″), 133.5 (quat., C-3), 133.1 (*C*H, C-9″), 130.8 (*C*H × 2, C-7″,11″), 130.5 (*C*H, C-α), 130.2 (*C*H × 2, C-4′,6′), 130.1 (quat., C-9), 126.1 (*C*H × 2, C-3′,5′), 123.9 (*C*H, d, *J* 9.1 Hz, C-7), 121.8 (*C*H × 2, C-8″,10″), 110.9 (*C*H, d, *J* 22.6 Hz, C-6), 105.9 (*C*H, d, *J* 23.7 Hz, C-4), 103.2 (*C*H, C-5″), 64.7 (CH_2, C-2″), 39.5 (CH_2, C-1″), 34.0 (CH_2, 3-CH_2), 15.6 (CH_3, SCH_3) and 10.9 (CH_3, 2-CH_3); **^{19}F {^{1}H} NMR** (376 MHz; $CDCl_3$) δ -114.0; ***m/z*** (ES^+) 593 ($[M+Na]^+$, 100%); **HRMS** *m/z* (ES^+) calcd. for $C_{31}H_{27}FN_4NaO_4S$ $[M+Na]^+$ requires 593.1635, found 593.1640; **CHN** Anal. calcd. for $C_{31}H_{27}FN_4O_4S$: C, 65.25; H, 4.77; N, 9.82. Found C, 65.30; H, 4.78; N, 9.89.

_N_-(((2-(2-((_Z_)-5-Fluoro-2-methyl-1-(4-(methylthio)benzylidene)-1_H_-inden-3-yl)acetamido)ethoxy)carbamoyl)-3-phenylsydnonimine, 230



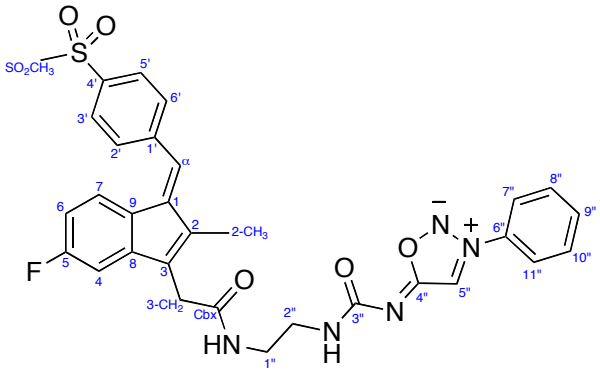
Amine **224** (100 mg, 0.26 mmol) and *N*-((4-nitrophenoxy)carbonyl)-3-phenylsydnonimine **218** (85 mg, 0.26) were dissolved in acetonitrile (10 mL) and heated under reflux for 24 h. The solvent was removed under reduced pressure and the residue purified by silica gel chromatography, eluting with dichloromethane and methanol (100: 0 to 90:10), to provide *N*-(((*2*-(*2*-((*Z*)-*5-fluoro-2-methyl-1*-(*4*-(*methylthio)benzylidene)-1H-inden-3-yl)acetamido)ethoxy)carbamoyl)-3-phenylsydnonimine* **230** (126 mg, 0.22 mmol, 86%) as a yellow solid: $\boldsymbol{R_f}$ 0.60 (90:10, CH_2Cl_2:MeOH, UV/cerium phosphomolybdate); **m.p.** 129-130 °C; $\boldsymbol{\nu_{max}}$ (thin film)/cm^{-1} 1632, 1601, 1548, 1467, 1365, 1293, 1168, 1092, 966; **^{1}H NMR** (400 MHz; d_6-DMSO) δ 8.27 (1H, s, C*H*-5″), 8.12 (1H, t, *J* 5.0 Hz, N*H*), 7.96 (2H, d, *J* 8.6 Hz, C*H*-7″,11″), 7.57-7.66 (3H, m, C*H*- 3′,5′,9″), 7.48-7.44 (2H, m, C*H*-8″,10″), 7.34-7.28 (3H, m, C*H*-7,2′,6′), 7.23 (1H, s, C*H*-α), 7.06 (1H, dd, *J* 9.4, 2.5 Hz, C*H*-4), 6.96 (1H, t, *J* 5.3 Hz, N*H*), C*H*), 6.70 (1H, m, C*H*-6), 3.40 (2H, s, 3-CH_2), 3.14 (4H, app. s, CH_2-1″, 2″), 2.51 (3H, s, SCH_3) and 2.15 (3H, s, 2-CH_3); **^{13}C NMR** (100 MHz; $CDCl_3$) 171.1 (quat., C-4″), 169.4 (quat., carboxyl), 162.7 (quat., d, *J* 242.9 Hz, C-5), 161.3 (quat., C-3″), 147.3 (quat., d, *J* 8.9 Hz, C-8), 139.4 (quat., C-1), 139.2 (quat., C-2), 138.3 (quat., C-4′), 134.0 (quat., C-6″), 133.0 (*C*H, C-9″), 132.9 (quat., C-3), 132.8 (quat., C-9), 130.4 (quat., C-1′), 130.5 (*C*H × 2, C-4′,6′), 130.1 (*C*H × 2, C-7″,11″), 129.8 (*C*H, C-α), 125.7 (*C*H × 2, C-3′,5′), 123.1 (*C*H, d, *J* 8.9 Hz, C-7), 122.3 (*C*H × 2, C-8″,10″), 111.3 (*C*H, d, *J* 22.8 Hz, C-6), 106.1 (*C*H, d, *J* 23.8 Hz, C-4), 102.5 (*C*H, C-5″), 41.1 (*C*H_2, × 2 C-1″,2″), 33.0 (*C*H_2, 3-CH_2), 15.4 (*C*H_3, SCH_3) and 10.6 (*C*H_3, 2-CH_3); **^{19}F {^{1}H} NMR** (376 MHz; $CDCl_3$) δ -114.5; ***m/z*** (ES^+) 592 ([M+Na]$^+$, 100%); **HRMS** *m/z* (ES^+) calcd. for $C_{31}H_{28}FN_5NaO_3S$ [M+Na]$^+$ requires 592.1795, found 592.1801; **CHN** Anal. calcd. for $C_{31}H_{28}FN_5O_3S$: C, 65.36; H, 4.95; N, 12.29. Found C, 65.39; H, 4.40; N, 12.32.

_N_-(((2-(2-((_Z_)-5-Fluoro-2-methyl-1-(4-(methylsulfonyl)benzylidene)-1_H_-inden-3-yl)acetamido)ethoxy)carbonyl)-3-phenylsydnonimine, 231



Alcohol **225** (100 mg, 0.25 mmol) and *N*-((4-nitrophenoxy)carbonyl)-3-phenylsydnonimine **218** (82 mg, 0.25) were dissolved in acetonitrile (10 mL) and heated under reflux for 24 h. The solvent was removed under reduced pressure and the residue purified by silica gel chromatography, eluting with dichloromethane and methanol (100: 0 to 90:10), to provide *N*-(((*2*-(*2*-((*Z*)-*5-fluoro-2-methyl-1*-(*4*-(*methylsulfonyl)benzylidene)-1H-inden-3-yl)acetamido)-ethoxy)carbonyl)-3-phenyl-sydnonimine* **231** (84 mg, 0.15 mmol, 49%) as a yellow solid: $\boldsymbol{R_f}$ 0.62 (90:10, CH_2Cl_2:MeOH, UV/cerium phosphomolybdate); **m.p.** 99-101 °C; $\boldsymbol{\nu_{max}}$ (thin film)/cm^{-1} 1660, 1603, 1590, 1468, 1369, 1308, 1280, 1199, 1148, 970; **^{1}H NMR** (400 MHz; $CDCl_3$) δ 8.06 (1H, s, C*H*-5″), 8.01 (2H, d, *J* 8.4 Hz, C*H*-3′,5′), 7.88-7.81 (2H, m, C*H*-7′, 11′), 7.74-7.66 (5H, m, C*H*-2′,6′,8″,9″,10″), 7.13 (1H, s, C*H*-α), 7.04 (1H, dd, *J* 8.4, 5.1, C*H*-7), 6.85 (1H, dd, *J* 8.8, 2.4 Hz, C*H*-4), 6.46 (1H, ddd, *J* 9.0, 8.8, 2.4 Hz, C*H*-6), 6.30 (1H, t, *J* 6.0 Hz, N*H*), 4.20 (2H, t, *J* 5.1 Hz, CH_2-2″), 3.59 (2H, q, *J* 6.0 Hz, CH_2-1″), 3.52 (2H, s, 3-CH_2), 3.14 (3H, s, SO_2CH_3) and 2.20 (3H, s, 2-CH_3); **^{13}C NMR** (100 MHz; $CDCl_3$) δ 175.3 (quat., C-4″), 169.5 (quat., carboxyl), 163.7 (quat., d, *J* 247.1 Hz, C-5), 161.4 (quat., C-3″), 146.9 (quat., d, *J* 8.6 Hz, C-8), 142.7 (quat., C-4′), 142.5 (quat., C-1′), 140.1 (quat., C-1), 138.9 (quat., C-2), 134.0 (quat., C-6″), 133.6 (*C*H, C-9″), 133.3 (quat., C-3), 130.9 (*C*H × 2, C-7″,11″), 130.5 (*C*H × 2, C-4′,6′), 129.7 (quat., C-9), 128.7 (*C*H × 2, C-3′,5′), 127.7 (*C*H, C-α), 123.9 (*C*H, d, *J* 9.3 Hz, C-7), 121.7 (*C*H × 2, C-8″,10″), 111.2 (*C*H, d, *J* 22.8 Hz, C-6), 106.5 (*C*H, d, *J* 23.7 Hz, C-4), 103.1 (*C*H, C-5″), 64.7 (CH_2, C-2″), 44.8 (CH_3, SO_2CH_3), 39.4 (CH_2, C-1″), 34.0 (CH_2, 3-CH_2) and 10.9 (CH_3, 2-CH_3); **^{19}F {^{1}H} NMR** (376 MHz; $CDCl_3$) δ -112.6; ***m/z*** (ES^+) 625 ($[M+Na]^+$, 100%); **HRMS** *m/z* (ES^+) calcd. for $C_{31}H_{27}FN_4NaO_6S$ $[M+Na]^+$ requires 625.1533, found 625.1535; **CHN** Anal. calcd. for $C_{31}H_{27}FN_4O_6S$: C, 61.78; H, 4.52; N, 9.30. Found C, 61.80; H, 4.55; N, 9.33.

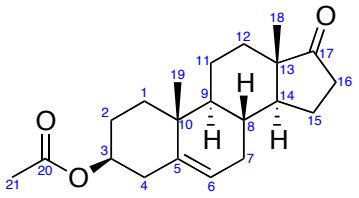
_N_-(((2-(2-((_Z_)-5-Fluoro-2-methyl-1-(4-(methylsulfonyl)benzylidene)-1_H_-inden-3-yl)acetamido)ethoxy)carbamoyl)-3-phenylsydnonimine, 232



Amine **226** (100 mg, 0.24 mmol) and *N*-((4-nitrophenoxy)carbonyl)-3-phenylsydnonimine **218** (79 mg, 0.24) were dissolved in acetonitrile (10 mL) and heated under reflux for 24 h. The solvent was removed under reduced pressure and the residue purified by silica gel chromatography, eluting with dichloromethane and methanol (100: 0 to 90:10), to provide *N*-(((*2*-(*2*-((*Z*)-*5-fluoro-2-methyl-1*-(*4*-(*methylsulfonyl)benzylidene)-1H-inden-3-yl)acetamido)ethoxy)-carbamoyl)-3-phenyl-sydnonimine* **232** (131 mg, 0.22 mmol, 90%) as a yellow solid: $\boldsymbol{R_f}$ 0.59 (90:10, CH_2Cl_2:MeOH, UV/cerium phosphomolybdate); **m.p.** 118-120 °C; $\boldsymbol{\nu_{max}}$ (thin film)/cm^{-1} 1730, 1650, 1626, 1530, 1469, 1303, 1145, 1087; **^{1}H NMR** (400 MHz; d_6-DMSO) δ 8.34 (1H, s, C*H*-5″), 8.12 (1H, t, *J* 5.0 Hz, N*H*), 8.01 (2H, d, *J* 8.3 Hz, C*H*-3′,5′), 7.79-7.66 (7H, m, C*H*-2′,6′,7″,8″,9″,10″,11″), 7.34 (1H, s, C*H*-α), 7.14-7.06 (2H, m, C*H*-7 and C*H*-4), 7.00 (1H, t, *J* 5.5 Hz, N*H*), 6.89 (1H, ddd, *J* 9.0, 8.6, 2.6 Hz, C*H*-6), 3.43 (2H, s, 3-CH_2), 3.29 (3H, s, SO_2CH_3), 3.16-3.13 (4H, m, CH_2-1″, 2″) and 2.18 (3H, s, 2-CH_3); **^{13}C NMR** (100 MHz; d_6-DMSO) 171.8 (quat., C-4″), 168.8 (quat., carboxyl), 162.6 (quat., d, *J* 249.1 Hz, C-5), 161.1 (quat., C-3″), 147.4 (quat., d, *J* 9.8 Hz, C-8), 141.5 (quat., C-4′), 141.1 (quat., C-1′), 140.1 (quat., C-1), 137.8 (quat., C-2), 134.1 (quat., C-3), 133.8 (quat., C-6″), 132.6 (*C*H, C-9″), 130.2 (*C*H × 2, C-4′,6′), 130.0 (*C*H × 2, C-7″,11″), 127.2 (*C*H × 2, C-3′,5′), 129.3 (quat., C-9), 128.3 (*C*H, C-α), 123.1 (*C*H, d, *J* 7.3 Hz, C-7), 122.1 (*C*H × 2, C-8″,10″), 110.3 (*C*H, d, *J* 23.2 Hz, C-6), 106.3 (*C*H, d, *J* 25.4 Hz, C-4), 102.3 (*C*H, C-5″), 44.3 (*C*H_3, SO_2CH_3), 39.8 (*C*H_2, × 2 C-1″, C-2″), 32.8 (*C*H_2, 3-CH_2) and 10.4 (*C*H_3, 2-CH_3); **^{19}F {^{1}H} NMR** (376 MHz; $CDCl_3$) δ -113.7; ***m/z*** (ES^+) 624 ([M+Na]$^+$, 100%); **HRMS** *m/z* (ES^+) calcd. for $C_{31}H_{28}FN_5NaO_5S$ [M+Na]$^+$ requires 624.1693, found 624.1700; **CHN** Anal. calcd. for $C_{31}H_{28}FN_5O_5S$: C, 61.89; H, 4.69; N, 11.64. Found C, 61.90; H, 4.72; N, 11.64.

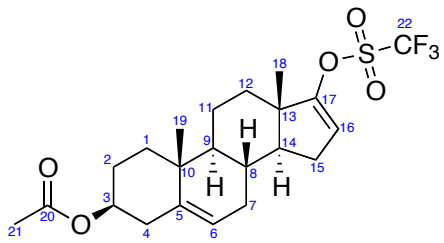
A.5 EXPERIMENTAL PROCEDURES FOR CHAPTER FOUR

(3*β*)-3-(Acetyloxy)-5-androsten-17-one, 243[402]



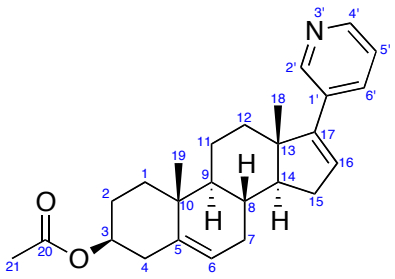
Boron trifluoride diethyl etherate (22 mg, 10 µL, 0.16 mmol) was added to a suspension of dehydroepiandrosterone **235** (1.00 g, 3.47 mmol) in dichloromethane (5 mL). Acetic anhydride (460 mg, 430 µL, 4.51 mmol) was added dropwise and the solution stirred for 4 h. Water (10 mL) was added and the biphasic mixture stirred for 30 min. The aqueous layer was extracted with dichloromethane (15 mL). The organic layers were combined and washed with aq. Sodium hydrogen carbonate solution (saturated, 15 mL), dried over Na_2SO_4. The mixture was filtered and the solvent removed under reduced pressure to provide (3*β*)-3-(acetyloxy)-5-androsten-17-one **243** (1.15 g, 3.47 mmol, quant.) as a white solid, which was used without any further purification: $\boldsymbol{R_f}$ 0.55 (20:80, EtOAc:cyclohexane, UV/cerium phosphomolybdate); **m.p.** (hexane) 171-172 °C, [Lit.[403] 172 °C]; $\boldsymbol{[\alpha]_D^{20}}$ -6.9 (*c* = 2.0, $CHCl_3$), [Lit.[403] -7.0, (*c* = 2.0, $CHCl_3$]; **^{1}H NMR** (300 MHz; $CDCl_3$) δ 5.41 (1H, d, *J* 5.0 Hz, C*H*-6), 4.66-4.55 (1H, m, C*H*-3), 2.51 (1H, dd, *J* 20.0, 9.0 Hz, CH_AH$_B$-16), 2.38-2.28 (2H, m, CH_2-1), 2.17-2.05 (2H, m, CH$_A$$H_B$-16, C$H_AH_B$-12), 2.03 (3H, s, C$H_3$-21), 2.01-1.92 (1H, m, CH_AH$_B$-15), 1.92-1.79 (3H, m, CH_AH$_B$-7, CH_AH$_B$-4, CH_AH$_B$-2), 1.76-1.40 (6H, m, CH$_A$$H_B$-15, CH$_A$$H_B$-12, C$H_2$-11, C*H*-8, CH$_A$$H_B$-2), 1.38-1.23 (2H, m, C*H*-9, CH$_A$$H_B$-7), 1.21-0.96 (5H, m, C*H*-14, CH$_A$$H_B$-4, C$H_3$-18 and C$H_3$-19), 0.88 (3H, s, C$H_3$-18); **^{13}C NMR** (100 MHz; $CDCl_3$) δ 221.1 (quat., C-17), 170.5 (quat., C-20), 139.9 (quat., C-5), 121.9 (*C*H, C-6), 73.7 (*C*H, C-3), 51.7 (*C*H, C-9), 49.5 (*C*H, C-14), 47.5 (quat., C-13), 40.0 (*C*H$_2$, C-4), 38.1 (*C*H$_2$, C-1), 36.8 (quat., C-10), 35.9 (*C*H$_2$, C-16), 31.5 (*C*H, C-8), 31.4 (*C*H$_2$, C-7), 30.8 (*C*H$_2$, C-12), 27.7 (*C*H$_2$, C-2), 21.9 (*C*H$_2$, C-15), 21.4 (*C*H$_3$, C-21), 20.3 (*C*H$_2$, C-11), 19.4 (*C*H$_3$, C-19), 13.6 (*C*H$_3$, C-18); ***m/z*** (ES$^+$) 353 ([M+Na]$^+$, 100%). The data were in agreement with the literature values.[402,403]

(3*β*)-Acetyloxyandrosta-5-16-dien-17-yl trifluoromethanesulfonate, 244[291]



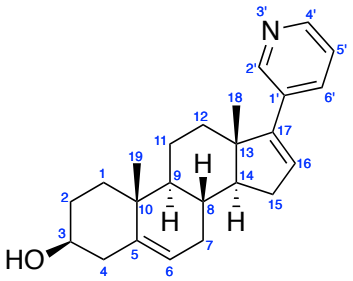
Trifluoromethanesulfonic anhydride (1.71 g, 1.02 mL, 6.06 mmol) was added dropwise to a solution of (3*β*)-3-(acetyloxy)-5-androsten-17-one **243** (2.00 g, 6.06 mmol) and 2,6-di-*tert*-butyl-4-methylpyridine (1.49 g, 7.27 mmol) and stirred at room temperature for 16 h. The suspension was filtered and the filtrate washed with water (4 mL), dried over $MgSO_4$. The mixture was filtered and the solvent adsorbed onto silica gel. Purification by silica gel chromatography, eluting with petroleum ether and dichloromethane (95:5 to 60:40) provided (3*β*)-acetyloxyandrosta-5-16-dien-17-yl trifluoromethanesulfonate **244** (1.64 g, 3.51 mmol, 58%) as an orange solid: $\boldsymbol{R_f}$ 0.67 (20:80, EtOAc:cyclohexane, UV/cerium phosphomolybdate); **m.p.** (hexane) 75-76 °C [Lit.[291] 75-76 °C]; $[\alpha]_D^{20}$ -34.8, (*c* = 1.0, $CHCl_3$); **^{1}H NMR** (300 MHz; $CDCl_3$) δ 5.58 (1H, dd, *J* 3.3, 1.7 Hz C*H*-16), 5.40-5.38 (1H, m, C*H*-6), 4.68-4.56 (1H, m, C*H*-3), 2.37-2.31 (2H, m, CH_2-1) 2.23 (1H, ddd, *J* 15.0, 6.3, 3.3 Hz, CH_AH$_B$-12), 2.03 (3H, s, CH_3-21), 2.02-1.98 (2H, m, CH_AH$_B$-15, CH$_A$$H_B$-12), 1.90-1.82 (2H, m, CH_AH$_B$-4, CH_AH$_B$-2), 1.71-1.44 (7H, m, CH$_A$$H_B$-15, C$H_2$-11, C*H*-9, C*H*-8, CH$_A$$H_B$-7, CH$_A$$H_B$-2), 1.18-1.08 (2H, m, C*H*-14, CH$_A$$H_B$-4), 1.05 (3H, s, C$H_3$-19), 0.99 (3H, s, C$H_3$-18); **^{13}C NMR** (100 MHz; $CDCl_3$) δ 170.5 (quat., C-20), 159.2 (quat., C-17), 140.1 (quat., C-5), 121.8 (*C*H, C-6), 117.3 (quat., C-22), 114.5 (*C*H_2, C-16), 73.7 (*C*H, C-3), 54.2 (*C*H, C-9), 50.3 (*C*H, C-14), 44.6 (quat., C-13), 38.1 (*C*H_2, C-1), 36.8 (*C*H_2, C-4), 36.7 (quat., C-10), 32.7 (*C*H_2, C-7), 30.5 (*C*H_2, C-12), 29.9 (*C*H, C-8), 28.6 (*C*H_2, C-15), 27.7 (*C*H_2, C-2), 21.5 (*C*H_3, C-21), 20.1 (*C*H_2, C-11), 19.2 (*C*H_3, C-19), 15.1 (*C*H_3, C-18); **^{19}F {^{1}H}** -74.1 (CF_3); ***m/z*** (ES$^+$) 485 ([M+H]$^+$, 100%). The data were in agreement with the literature values.[291]

3*β*-Acetoxy-17-(3-pyridyl)androsta-5,16-diene (abiraterone acetate), 246[291]



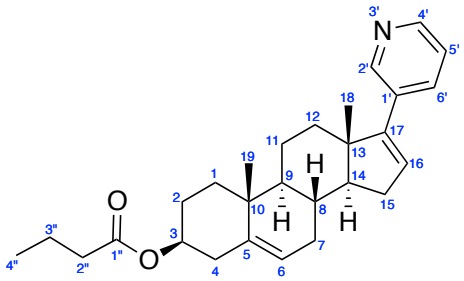
Diethyl(3-pyridyl)borane (974 mg, 6.66 mmol) was added to a solution of vinyl triflate **244** (2.00 g, 4.33 mmol) in THF (22 mL) containing *bis*(triphenylphosphine)palladium (II) chloride (60 mg, 0.09 mmol). Aqueous sodium carbonate (2 M, 8.6 mL) was added and the solution was stirred at 80 °C for 1 h. The biphasic mixture was partitioned between diethyl ether and water (1:1, 150 mL). The organic layer was separated and dried over Na_2CO_3. The mixture was filtered through a plug of silica gel and adsorbed onto silica gel. Purification by silica gel chromatography, eluting with petroleum ether and diethyl ether (85:15 to 40:60) provided 3*β*-acetoxy-17-(3-pyridyl)androsta-5,16-diene **246** (abiraterone acetate) **246** (1.47 g, 3.76 mmol, 87%) as a white solid: $\boldsymbol{R_f}$ 0.35 (20:80, EtOAc:cyclohexane, UV/cerium phosphomolybdate); **m.p.** 144-146 °C [Lit.[291] 144-145 °C]; $[\boldsymbol{\alpha}]_{\mathbf{D}}^{\mathbf{20}}$-41.4, (*c* = 1.0, $CHCl_3$); **^{1}H NMR** (300 MHz; $CDCl_3$) δ 8.58 (1H, d, *J* 1.7 Hz, C*H*-2′), 8.41 (1H, dd, *J* 5.0, 1.8 Hz, C*H*-6′), 7.60 (1H, ddd, *J* 8.0, 1.7, 1.8 Hz, C*H*-4′), 7.17 (1H, dd, *J* 8.0, 5.0 Hz, CH-5′), 5.95-5.94 (1H, m, C*H*-16), 5.37 (1H, d, *J* 5.7 Hz, C*H*-6), 4.62-4.51 (1H, m, C*H*-3), 2.39-2.29 (2H, m, CH_2-1), 2.22 (1H, ddd, *J* 15.8, 6.4, 3.5 Hz, CH_AH$_B$-12), 2.06-1.97 (2H, m, CH$_A$$H_B$-12, C$H_AH_B$-7), 1.97 (3H, s, C$H_3$-21), 1.84-1.77 (2H, m, CH_AH$_B$-2, CH_AH$_B$-4), 1.76-1.38 (8H, m, CH$_A$$H_B$-2, CH$_A$$H_B$-7, C*H*-8, C*H*-9, C$H_2$-11,15), 1.18-0.99 (2H, m, CH$_A$$H_B$-4, C*H*-14), 1.03 (3H, s, CH_3-19), 1.00 (3H, s, CH_3-18); **^{13}C NMR** (100 MHz; $CDCl_3$) δ 170.4 (quat., C-20), 151.6 (quat., C-17), 147.7 (quat. × 2, C-2′,6′), 140.0 (quat., C-5), 133.7 (*C*H, C-4′), 132.9 (quat., C-1′), 129.5 (*C*H_2, C-16), 123.0 (*C*H, C-5′), 122.3 (*C*H, C-6), 73.8 (*C*H, C-3), 57.4 (*C*H, C-9), 50.2 (*C*H, C-14), 47.3 (quat., C-13), 38.1 (*C*H_2, C-1), 36.9 (*C*H_2, C-4), 36.8 (quat., C-10), 35.2 (*C*H_2, C-7), 31.8 (*C*H_2, C-12), 31.5 (*C*H_2, C-15), 30.4 (*C*H, C-8), 27.7 (*C*H_2, C-2), 21.4 (*C*H_3, OAc), 20.8 (*C*H_2, C-11), 19.2 (*C*H_3, C-19), 16.6 (*C*H_3, C-18), ***m/z*** (ES$^+$) 414 ([M+Na]$^+$, 100%). The data were in agreement with the literature values.[291]

17-(3-Pyridyl)androsta-5,16-dien-3*β*-ol (abiraterone), 106[291]



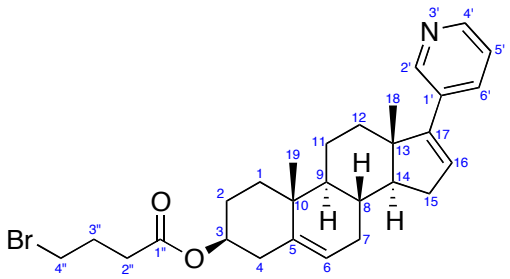
KOH (10% *w/v* in MeOH, 3.4 mL) was added to a solution of abiraterone acetate **246** (500 mg, 1.28 mmol) in MeOH (10 mL) and the resultant solution stirred for 1 hour. The suspension was added to water (50 mL), neutralised with aq. HCl (2 N) and rebasified with aq. sodium hydrogen carbonate solution (saturated). The aqueous froth was extracted with chloroform (3 × 75 mL), dried over Na_2SO_4. The mixture was filtered and the solvent evaporated under reduced pressure, to afford 17-(3-pyridyl)androsta-5,16-dien-3*β*-ol (abiraterone) **106** (446 mg, 1.28 mmol, quant.) as a white solid, which was used without any further purification: **m.p.** 228-230 °C [Lit.[291] 228-229 °C]; $\boldsymbol{R_f}$ 0.29 (50:50, EtOAc:Cyclohexane, UV/cerium phosphomolybdate); $\boldsymbol{[\alpha]_D^{20}}$ -47.6, (*c* = 1.0, $CHCl_3$); **^{1}H NMR** (300 MHz; $CDCl_3$) δ 8.61 (1H, d, *J* 1.9 Hz, C*H*-2′), 8.45 (1H, dd, *J* 4.9, 1.6 Hz, C*H*-6′), 7.64 (1H, ddd, *J* 7.9, 1.9, 1.6, C*H*-4′), 7.21 (1H, dd, *J* 7.9, 4.9 Hz, C*H*-5′), 5.99 (1H, d, *J* 3.8, 1.2 Hz, C*H*-16), 5.41-5.37 (1H, m, C*H*-6), 3.59-3.49 (1H, m, C*H*-3), 2.37-2.20 (3H, m, CH_2-1, CH_AH$_B$-2), 2.13-1.99 (3H, m, CH_2-12, CH_AH$_B$-7), 1.90-1.81 (2H, m, CH$_A$$H_B$-2, C$H_AH_B$-4), 1.79-1.72 (1H, m, C*H*-8), 1.72-1.46 (6H, m, CH$_A$$H_B$-7, C*H*-9, C$H_2$-11, C$H_2$-15), 1.15-1.09 (2H, m, CH$_A$$H_B$-4, C*H*-7). 1.07 (3H, s, CH_3-19), 1.04 (3H, s, CH_3-18); **^{13}C NMR** (100 MHz; $CDCl_3$) δ 151.7 (quat., C-17), 147.9 (quat. × 2, C-2′,6′), 141.2 (quat., C-5), 133.7 (*C*H, C-4′), 133.0 (quat., C-1′), 129.3 (*C*H$_2$, C-16), 123.0 (*C*H, C-5′), 121.6 (*C*H, C-6), 71.7 (*C*H, C-3), 57.6 (*C*H, C-9), 50.4 (*C*H, C-14), 47.4 (quat., C-13), 42.3 (*C*H$_2$, C-4), 37.2 (*C*H$_2$, C-1), 36.7 (quat., C-10), 35.3 (*C*H$_2$, C-7), 31.8 (*C*H$_2$, C-12), 31.7 (*C*H$_2$, C-2), 31.5 (*C*H$_2$, C-15), 30.5 (*C*H, C-8), 20.9 (*C*H$_2$, C-11), 19.4 (*C*H$_3$, C-19), 16.6 (*C*H$_3$, C-18); ***m/z*** (ES$^+$) 372 ([M+Na]$^+$, 100%). The data were in agreement with the literature values.[291]

17-(3-Pyridyl)androsta-5,16-dien-3*β*-ol 3-butanoate, 250



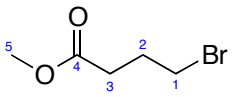
Butyric acid **250** (25 mg, 26 µL, 0.17 mmol) and DMAP (20 mg, 0.14 mmol) were added to a solution of abiraterone **106** (50 mg, 0.14 mmol) in dichloromethane (7 mL). EDCI.HCl (40 mg, 0.210 mmol) was added in a single portion and the solution stirred for 4 h. The solution was washed with water (5 mL) and dried over $MgSO_4$. The mixture was filtered and the solvent adsorbed onto silica gel. Purification by silica gel chromatography, eluting with ethyl acetate and petroleum ether (15:85 to 25:75), provided *17-(3-pyridyl)androsta*-5,16-*dien-3β-ol 3-butanoate* **250** (58 mg, 0.14, 97%) as a white solid: $\boldsymbol{R_f}$ 0.74 (50:50, EtOAc:cyclohexane, UV/cerium phosphomolybdate); **m.p.** 104-105 °C; $[\alpha]_D^{20}$ -14.8, (*c* = 1.0, $CHCl_3$); $\boldsymbol{\nu_{max}}$ (thin film)/cm^{-1} 1765, 1640, 1560, 1440, 1225, 1060, 751; **^{1}H NMR** (300 MHz; $CDCl_3$) δ 8.61 (1H, d, *J* 1.9 Hz, C*H*-2′), 8.45 (1H, dd, *J* 4.8, 1.5 Hz, C*H*-6′), 7.65 (1H, dt, *J* 8.0, 1.9 Hz, C*H*-4′), 7.22 (1H, dd, *J* 8.0, 4.8 Hz, C*H*-5′), 5.99 (1H, dd, *J* 3.2, 1.7 Hz, C*H*-16), 5.41 (1H, d, *J* 5.2 Hz, C*H*-6), 4.66-4.59 (1H, m, C*H*-3), 2.36-2.31 (2H, m, CH_2-1), 2.28-2.24 (3H, m, CH_2-2″, CH_AH_B-12), 2.09-2.01 (2H, m, CH_AH_B-12, CH_AH_B-7), 1.88-1.84 (2H, m, CH_AH_B-2, CH_AH_B-4), 1.78-1.55 (9H, m, CH_AH_B-2, CH_2-3″, C*H*-8,9, CH_2-11,15), 1.48 (1H, td, *J* 24.9, 12.4, 5.2 Hz, CH_AH_B-7), 1.27-1.12 (2H, m, CH_AH_B-4, C*H*-14), 1.08 (3H, s, CH_3-19), 1.04 (3H, s, CH_3-18) and 0.94 (3H, t, *J* 7.4 Hz, CH_3-4″); **^{13}C NMR** (100 MHz; $CDCl_3$) δ 173.2 (quat., C-1″), 151.6 (quat., C-17), 147.7 (*C*H, C-2′), 147.7 (*C*H, C-6′), 140.1 (quat., C-5), 133.8 (*C*H, C-4′), 133.0 (quat., C-1′), 129.3 (*C*H, C-16), 123.1 (*C*H, C-5′), 122.2 (*C*H, C-6), 73.6 (*C*H, C-3), 57.5 (*C*H, C-9), 50.3 (*C*H, C-14), 47.3 (quat., C-13), 38.2 (CH_2, C-1), 36.9 (CH_2, C-4), 36.8 (quat., C-10), 35.2 (CH_2, C-7), 31.8 (CH_2, C-12), 31.5 (CH_2, C-2″), 31.5 (CH_2, C-15), 30.4 (*C*H, C-8), 27.7 (CH_2, C-2), 20.8 (CH_2, C-11), 19.3 (CH_3, C-19), 18.6 (CH_2, C-3″), 16.6 (CH_3, C-18) and 13.7 (CH_3, C-4″); ***m/z*** (ES^+) 442 ($[M+Na]^+$, 100%); **HRMS** *m/z* (ES^+) calcd. for $C_{28}H_{37}NNaO_2$ $[M+Na]^+$ requires 442.2722, found 442.2725; **CHN** Anal. calcd. For $C_{28}H_{37}NO_2$: C, 80.15; H, 8.89; N, 3.34. Found C, 80.22; H, 8.95; N, 3.35.

17-(3-Pyridyl)androsta-5,16-dien-3*β*-ol 3-(4-bromo)butanoate, 257



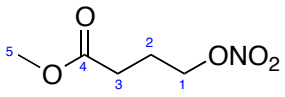
4-Bromobutyric acid **256** (53 mg, 0.32 mmol) and DMAP (35 mg, 0.29 mmol) were added to a solution of abiraterone **106** (100 mg, 0.29 mmol) in chloroform (7 mL). EDCI.HCl (36 mg, 0.22 mmol) was added in a single portion and the solution stirred for 4 h. The solution was washed with water and dried over $MgSO_4$. The mixture was filtered and the solvent evaporated to yield *17-(3-pyridyl)androsta*-5,16-*dien-3β-ol 3-(4-bromo)butanoate* **257** (130 mg, 0.26, 92%) as an off white solid: $\boldsymbol{R_f}$ 0.61 (50:50, EtOAc:cyclohexane, UV/cerium phosphomolybdate); **m.p.** 94-98 °C; $[\boldsymbol{\alpha}]_{\boldsymbol{D}}^{\boldsymbol{20}}$+4.0, (*c* = 1.0, $CHCl_3$); $\boldsymbol{\nu_{max}}$ (thin film)/cm^{-1} 3040, 2939, 1772, 1727, 1650, 1601, 1464, 1373, 1259, 1200, 1129, 1024, 996, 798, 735; $^{\mathbf{1}}$**H NMR** (300 MHz; $CDCl_3$) δ 8.52 (1H, s, C*H*-2′), 8.45 (1H, d, *J* 4.1 Hz, C*H*-6′), 7.64 (1H, dt, *J* 8.4, 1.7 Hz, C*H*-4′), 7.21 (1H, dd, *J* 7.9, 4.8 Hz, C*H*-5′), 5.98 (1H, dd, *J* 2.7, 1.5 Hz, C*H*-16), 5.42 (1H, d, *J* 4.9 Hz, C*H*-6), 4.60-4.54 (1H, m, C*H*-3), 3.44 (2H, t, *J* 7.1 Hz, CH_2-4″), 2.47 (2H, t, *J* 7.3 Hz, CH_2-2″), 2.35-2.30 (2H, m, CH_2-1), 2.28-2.24 (1H, m, CH_AH_B-12), 2.16 (2H, pentet, *J* 7.1 Hz, CH_2-3″), 2.03-1.96 (2H, m, CH_AH_B-7, CH_AH_B-12), 1.83-1.78 (2H, m, CH_AH_B-2, CH_AH_B-4), 1.73-1.39 (8H, m, CH_AH_B-2, CH_AH_B-7, C*H*-8,9, CH_2-11,15) 1.09-1.04 (2H, m, CH_AH_B-4, C*H*-14), 1.04 (3H, s, CH_3-19) and 1.00 (3H, s, CH_3-18); $^{\mathbf{13}}$**C NMR** (100 MHz; $CDCl_3$) δ 172.0 (quat., C-1″), 151.7 (quat., C-17), 147.9 (*C*H, C-2′), 147.9 (*C*H, C-6′), 139.9 (quat., C-5), 133.7 (*C*H, C-4′), 132.9 (quat., C-1′), 129.2 (*C*H, C-16), 123.0 (*C*H, C-5′), 122.4 (*C*H, C-6), 74.1 (*C*H, C-3), 57.4 (*C*H, C-9), 50.2 (*C*H, C-14), 47.3 (quat., C-13), 38.1 (CH_2, C-1), 36.9 (CH_2, C-4), 36.8 (quat., C-10), 35.2 (CH_2, C-7), 32.8 (CH_2, C-2″), 32.8 (CH_2, C-4″), 31.8 (CH_2, C-12), 31.5 (CH_2, C-15), 30.4 (*C*H, C-8), 27.8 (CH_2, C-3″), 27.7 (CH_2, C-2), 20.8 (CH_2, C-11), 19.2 (CH_3, C-19), 16.6 (CH_3, C-18); ***m/z*** (ES^+) 520 ($[M+Na]^+$, 100%); **HRMS** *m/z* (ES^+) calcd. for $C_{28}H_{36}BrNNaO_2$ $[M+Na]^+$ requires 520.1827, found 520.1825.

Methyl 4-bromobutanoate, 259[404]



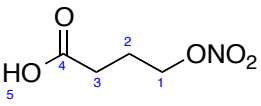
Acetyl chloride (576 mg, 550 µL, 7.34 mmol) was added to a solution of 4-bromobutyric acid **256** (5.00 g, 30.0 mmol) in methanol (40 mL) cooled to 0 °C. The resulting solution was allowed to warm to room temperature and stirred for 18 h. The solution was evaporated under reduced pressure to yield methyl 4-bromobutanoate **259** (5.40 g, 30.0 mmol quant.) as a colourless oil, which was used without any further purification: **^{1}H NMR** (500 MHz; $CDCl_3$) δ 3.56 (3H, s, C*H*$_3$-5), 3.35 (2H, t, *J* 6.6 Hz, C*H*$_2$-1), 2.38 (2H, t, *J* 7.6 Hz, C*H*$_2$-3), 2.04 (2H, tt, *J* 7.4, 6.6, C*H*$_2$-2); **^{13}C NMR** (125 MHz; $CDCl_3$) δ 172.7 (quat., C-4), 51.5 (*C*H$_3$, C-5), 32.7 (*C*H$_2$, C-1), 32.0 (*C*H$_2$, C-3), 27.7 (*C*H$_2$, C-2); ***m/z*** (ES$^+$) 203 ([M+Na]$^+$, 100%). The data were in agreement with the literature values.[405]

Methyl 4-(nitrooxy)butanoate, 260[406]



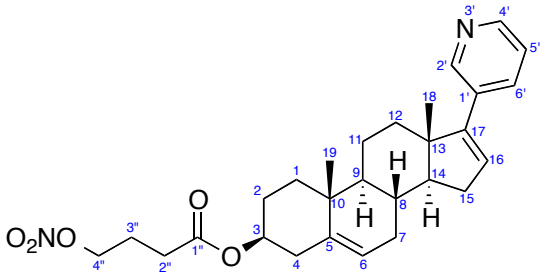
Silver nitrate (7.0 g, 41.3 mmol) was added to a solution of methyl 4-bromobutanoate **259** (3.0 g, 16.5 mmol) in dry acetonitrile (60 mL). The reaction was heated to 80 °C for 4 h, protected from light. The reaction mixture was filtered through Celite and the solvent removed under reduced pressure. The crude residue suspended in ethyl acetate (50 mL) and filtered through a silica plug. The organic layer was washed with water (100 mL) and brine (100 mL), dried over Na_2SO_4, filtered and the solvent removed under reduced pressure to 4-(nitrooxy)butanoate **260** (2.6 g, 15.9 mmol, 97%) as a pale yellow oil, which was used without any further purification: **^{1}H NMR** (300 MHz; $CDCl_3$) δ 3.56 (3H, s, *CH*$_3$-5), 3.35 (2H, t, *J* 6.6 Hz, C*H*$_2$-1), 2.38 (2H, t, *J* 7.4 Hz, C*H*$_2$-3), 2.04 (2H, tt, *J* 7.4, 6.6 Hz, C*H*$_2$-2); **^{13}C NMR** (100 MHz; $CDCl_3$) δ 173.1 (quat., C-4), 72.4 (*C*H$_2$, C-1), 52.2 (*C*H$_3$, C-5), 30.3 (*C*H$_2$, C-3), 22.6 (*C*H$_2$, C-2); ***m/z*** (ES$^+$) 186 ([M+Na]$^+$, 100%). The data were in agreement with the literature values.[406]

4-(Nitrooxy)butanoic acid, 258[406]



LiOH (2 N, 6.0 mL) was added to a solution of methyl 4-(nitrooxy)butanoate **260** (1.0 g, 6.1 mmol) in methanol (25 mL) cooled to 0 °C. The suspension was stirred overnight at 5 °C. The pH of the solution was adjusted to pH = 2 with HCl (2 N) and the solution concentrated under reduced pressure. The aqueous layer was extracted with dichloromethane (50 mL). The organic layer was separated and dried over Na_2SO_4, filtered and the solvent removed under reduced pressure to yield 4-(nitrooxy)butanoic acid **258** (700 mg, 4.69 mmol, 77%) as a colourless oil, which was used without any further purification. **^{1}H NMR** (300 MHz; $CDCl_3$) δ 8.88 (1H, s, *br*, CO_2H-5), 4.52 (2H, t, *J* 6.3 Hz, CH_2-1), 2.52 (2H, t, *J* 7.1 Hz, CH_2-3), 2.07 (2H, tt, *J* 7.1, 6.3, CH_2-2); **^{13}C NMR** (100 MHz; $CDCl_3$) δ 178.3 (quat., C-4), 72.2 (CH_2, C-1), 30.3 (CH_2, C-3), 22.4 (CH_2, C-2); ***m/z*** (ES^-) 148 ($[M-H]^-$, 100%). The data were in agreement with the literature values.[406]

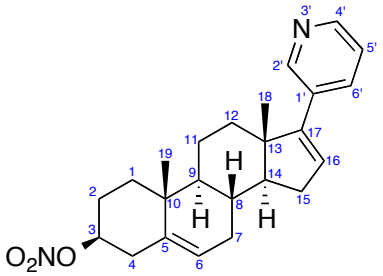
17-(3-Pyridyl)androsta-5,16-dien-3*β*-ol 3-(4-nitrooxy)butanoate, 251



4-(Nitrooxy)butanoic acid **258** (30 mg, 0.20 mmol) and DMAP (21 mg, 0.17 mmol) were added to a solution of abiraterone **106** (60 mg, 0.17 mmol) in dichloromethane (7 mL). EDCI.HCl (40 mg, 0.210 mmol) was added in a single portion and the solution was stirred for 6 h. The solution was washed with water and dried over $MgSO_4$. The mixture was filtered and the solvent adsorbed onto silica gel. Purification by silica gel chromatography, eluting with ethyl acetate and petroleum ether (15:85 to 25:75), provided *17-(3-pyridyl)androsta*-5,16-*dien-3β-ol 3-(4-nitrooxy)butanoate* **251** (78 mg, 0.16 mmol, 95%) as a white solid: $\boldsymbol{R_f}$ 0.70 (90:10, CH_2Cl_2:acetone, UV/cerium phosphomolybdate); **m.p.** 68-70 °C; $[\boldsymbol{\alpha}]_{\mathbf{D}}^{\mathbf{20}}$-8.3, (*c* = 1.0, $CHCl_3$); $\boldsymbol{\nu_{max}}$ (thin film)/cm^{-1} 2937, 1730, 1630, 1441, 1410, 1375, 1280, 1177, 1026, 930, 868, 797, 711;**^{1}H NMR** (300 MHz; $CDCl_3$) δ 8.63 (1H, s, *br*, C*H*-2′), 8.47 (1H, s, *br*, C*H*-6′), 7.71 (1H,

dt, *J* 8.1, 1.6 Hz, C*H*-4′), 7.28 (1H, dd, *J* 8.1, 5.0 Hz, C*H*-5′), 6.02 (1H, dd, *J* 3.1, 1.7 Hz, C*H*-16), 5.41 (1H, d, *J* 5.2 Hz, C*H*-6), 4.69-4.59 (1H, m, C*H*-3), 4.51 (2H, t, *J* 6.3 Hz, CH_2-4″), 2.52-2.23 (6H, m, CH_2-1, CH_2-2″, CH_2-12), 2.11-2.01 (5H, m, CH_2-3″, CH_AH$_B$-7, CH_2-15), 1.92-1.83 (2H, m, CH_AH$_B$-2, CH_AH$_B$-4), 1.80-1.44 (6H, m, CH$_A$*H*$_B$-2, CH$_A$*H*$_B$-7, C*H*-8, 9, CH_2-11,), 1.25-1.10 (2H, m, CH$_A$*H*$_B$-4, C*H*-14,), 1.08 (3H, s, CH_3-19) and 1.04 (3H, s, CH_3-18); **^{13}C NMR** (100 MHz; $CDCl_3$) δ 171.7 (quat., C-1″), 151.3 (quat., C-17), 146.9 (*C*H, C-2′), 146.9 (*C*H, C-6′), 139.9 (quat., C-5), 134.5 (*C*H, C-4′), 133.0 (quat., C-1′), 129.9 (*C*H, C-16), 123.4 (*C*H, C-5′), 122.4 (*C*H, C-6), 74.3 (*C*H, C-3), 72.1 (*C*H_2, C-4″), 57.5 (*C*H, C-9), 50.2 (*C*H, C-14), 47.4 (quat., C-13), 38.1 (*C*H_2, C-1), 36.9 (*C*H_2, C-4), 36.8 (quat., C-10), 35.2 (*C*H_2, C-7), 31.5 (*C*H_2, C-2″), 31.9 (*C*H_2, C-12), 31.5 (*C*H_2, C-15), 30.4 (*C*H, C-8), 27.7 (*C*H_2, C-2), 22.4 (*C*H_2, C-3″), 20.8 (*C*H_2, C-11), 19.3 (*C*H_3, C-19), 16.6 (*C*H_3, C-18); ***m/z*** (ES$^+$) 503 ([M+Na]$^+$, 100%); **HRMS** *m/z* (ES$^+$) calcd. for $C_{28}H_{36}N_2NaO_5$ [M+Na]$^+$ requires 503.2522, found 503.2530; **CHN** Anal. calcd. For $C_{28}H_{36}N_2O_5$: C, 69.98; H, 7.55; N, 5.83. Found C, 70.03; H, 7.59; N, 5.88.

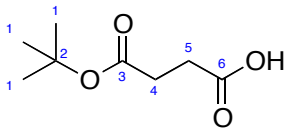
17-(3-Pyridyl)androsta-5,16-dien-3*β*-nitrate, 252



Zinc nitrate hexahydrate (85 mg, 0.29 mmol) was added to a solution of abiraterone **206** (100 mg, 0.286 mmol) in acetonitrile and dichloromethane (1:1, 10 mL). EDCI.HCl (110 mg, 0.57 mmol) was added in a single portion and the solution stirred for 18 h. The solution was washed with water and dried over $MgSO_4$. The mixture was filtered and the solvent adsorbed onto silica gel. Purification by silica gel chromatography, eluting with ethyl acetate and petroleum ether (15:85 to 25:75), provided *17-(3-pyridyl)androsta*-5,16-*dien-3β-nitrate* **252** (39 mg, 0.10 mmol, 35%, 88% b.r.s.m) as a white solid: R_f 0.77 (90:10, EtOAc:PE, UV/cerium phosphomolybdate); **m.p.** 91-94 °C; $[\alpha]_D^{20}$-9.9, (*c* = 1.0, $CHCl_3$); ν_{max} (thin film)/cm^{-1} 1729, 1669, 1627, 1464, 1378, 1279, 1176, 1012, 867, 748; **^{1}H NMR** (300 MHz; $CDCl_3$) δ 8.62 (1H, d, *J* 1.5 Hz, C*H*-2′), 8.46 (1H, dd, *J* 4.8, 1.3 Hz, C*H*-6′), 7.64 (1H, dt, *J* 7.8, 1.8 Hz, C*H*-4′), 7.22 (1H, dd, *J* 7.8, 4.8 Hz, C*H*-5′), 6.00 (1H, dd, *J* 3.2, 1.7 Hz, C*H*-16), 5.49 (1H, d, *J* 5.4 Hz, C*H*-6), 4.85-4.78 (1H, m, C*H*-3), 2.49 (1H, ddd, *J* 13.0, 4.9, 2.2 Hz, CH_AH$_B$-11), 2.45-2.38 (1H, m, CH$_A$*H*$_B$-11), 2.27 (1H, ddd, *J* 15.9, 6.5, 3.3 Hz, CH_AH$_B$-12), 2.12-1.93 (4H, m, CH_AH$_B$-2, CH_AH$_B$-4, CH_AH$_B$-7,

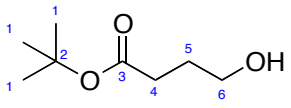
CH_AH_B-12), 1,82-1.56 (6H, m, CH_AH_B-2, C*H*-9, CH_2-11, CH_2-15), 1.49 (1H, ddd, *J* 24.0, 12.0, 5.1 Hz, CH_AH_B-7), 1.26-1.18 (2H, m, CH_AH_B-4, C*H*-8), 1.13-1.09 (1H, m, C*H*-14), 1.08 (CH_3, s, CH_3-19) and 1.05 (CH_3, s, CH_3-18); **^{13}C NMR** (100 MHz; $CDCl_3$) δ 151.6 (quat., C-17), 147.9 (quat. × 2, C-2′,6′), 138.8 (quat., C-5), 133.7 (*C*H, C-4′), 132.9 (quat., C-1′), 129.3 (CH_2, C-16), 123.6 (*C*H, C-5′), 123.0 (*C*H, C-6), 83.3 (*C*H, C-3), 57.4 (*C*H, C-9), 50.2 (*C*H, C-14), 47.3 (quat., C-13), 36.9 (CH_2, C-4), 36.7 (quat., C-10), 36.3 (CH_2, C-1), 35.1 (CH_2, C-7), 31.8 (CH_2, C-12), 31.5 (CH_2, C-15), 30.3 (*C*H, C-8), 25.9 (CH_2, C-2), 20.8 (CH_2, C-11), 19.2 (CH_3, C-19), 16.6 (CH_3, C-18); ***m/z*** (ES^+) 417 ($[M+Na]^+$, 100%); **HRMS** *m/z* (ES^+) calcd. for $C_{24}H_{30}N_2NaO_3$ $[M+Na]^+$ requires 417.2154, found 417.2150.

Succinic acid mono-*tert*-butyl ester, 264[407]



Succinic anhydride **263** (10 g, 100 mmol), *N*-hydroxysuccinimide (3.34 g, 0.30 mmol), and DMAP (1.17 g, 1.00 mmol) were dissolved in toluene (50 mL). *tert*-Butanol (11.7 mL, 124 mmol) and Et_3N (3.03 g, 4.17 mL, 30.0 mmol) were added sequentially. The suspension was heated under reflux for 24 h. The solution was cooled and diluted with EtOAc (50 mL) and was washed with citric acid (10% *w/v*, 100 mL) and brine (100 mL), dried over Na_2SO_4, and concentrated to give a brown solid. The solid was recrystallised with ether and petroleum ether (10:90) at -20 °C to give succinic acid mono-*tert*-butyl ester **264** (13.3 g, 76.4 mmol, 77%) as fawn crystals: **m.p.** 50-52 °C (PE) [Lit.[408] 49-52 °C]; **^{1}H NMR** (500 MHz; $CDCl_3$) δ 11.30 (1H, s, *br*, CO_2H), 2.60 (2H, t, *J* 6.0 Hz, CH_2-5), 2.50 (2H, t, *J* 6.0 Hz, CH_2-4), 1.42 (9H, s, 3 × CH_3-1); **^{13}C NMR** (100 MHz; $CDCl_3$) δ 178.7 (quat., C-6), 171.4 (quat., C-3), 81.0 (quat., C-2), 30.0 (CH_2), 29.9 (CH_2), 28.1 (CH_3 × 3, C-1); ***m/z*** (ES^+) 175 ($[M+H]^+$, 100%). The data were in agreement with the literature values.[407,408]

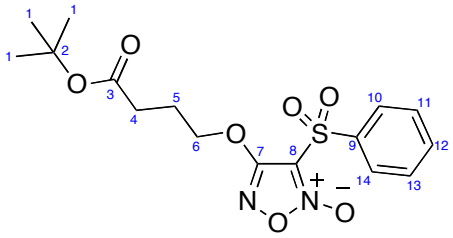
tert-Butyl 4-hydroxybutanoate, 262[409]



$BH_3.Me_2S$ (2.0 M in THF, 6.6 mL, 13.2 mmol) was added dropwise to a solution of carboxylic acid **264** (2.12 g, 12.2 mmol) in dry THF (20 mL) cooled to 0° C. The solution was allowed to

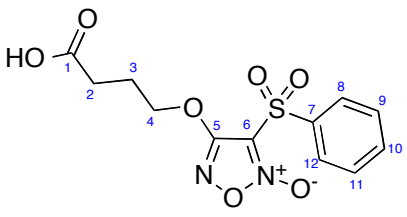
warm to room temperature and stirred for 24 h. EtOAc (100 mL) was added and the organic layer separated and washed with water (70 mL) and brine (70 mL), dried over $MgSO_4$, filtered and the solvent removed under reduced pressure to give *tert*-butyl 4-hydroxybutanoate **262** (1.92 g, 12.2 mmol, quant.), which was used without any further purification as a pale yellow oil: **^{1}H NMR** (500 MHz; $CDCl_3$) δ 3.65 (2H, t, *J* 6.1 Hz, CH_2-4), 2.33 (2H, t, *J* 7.3 Hz, CH_2-4), 2.17 (1H, s, *br*, O*H*), 1.82 (2H, tt, *J* 7.7, 7.4, CH_2-5) and 1.44 (9H, s, 3 × CH_3-1); **^{13}C NMR** (100 MHz; $CDCl_3$) δ 173.5 (quat., C-3), 80.5 (quat., C-2), 62.2 (*C*H_2, C-6), 32.4 (*C*H_2, C-4), 28.1 (*C*H_3 × 3, C-1) and 27.8 (*C*H_2, C-5); ***m/z*** (ES$^+$) 183 ([M+Na]$^+$, 100%). The data were in agreement with the literature values.[409]

4-(4-(*tert*-Butoxy)-4-oxobutoxy)-3-(phenylsulfonyl)-1,2,5-oxadiazole 2-oxide, 265



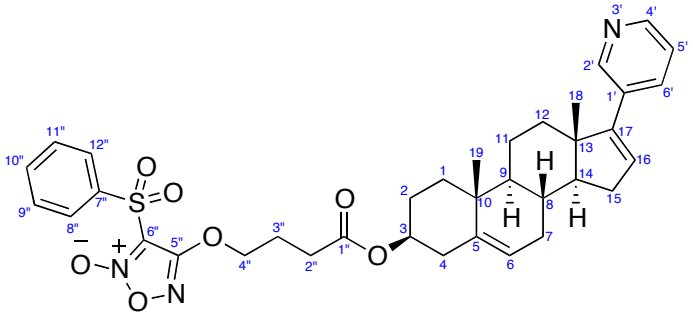
DBU (971 μL, 6.00 mmol) was added to a solution of *tert*-butyl 4-hydroxybutanoate **262** (480 mg, 3.00 mmol) in dichloromethane (15 mL) and the solution was stirred vigorously. *Bis*(phenylsulfonyl)-1,2,5-oxadiazole 2-oxide **53** (732 mg, 2.00 mmol) was added and stirring continued for 2 h. The reaction mixture was washed with distilled water (20 mL) and with HCl (2 N, 2 × 15 mL) and brine (20 mL). The organic layer was dried over Na_2SO_4, filtered and the solvent removed under reduced pressure to give the crude product. This product was purified by silica gel chromatography, eluting with dichloromethane, to give *4-(4-(tert-butoxy)-4-oxobutoxy)-3-(phenylsulfonyl)*-1,*2,5-oxadiazole 2-oxide* **265** (595 mg, 1.71 mmol, 86%) as a colourless oil which solidified on standing; ***R$_f$*** 0.76 (30:70, EtOAc:PE, UV/$KMnO_4$); **m.p.** 95-97 °C; **ν_{max}** (thin film)/cm^{-1} 2098, 1616, 1550, 1449, 1358, 1166; **^{1}H NMR** (500 MHz; $CDCl_3$) δ 8.03 (2H, d, *J* 8.7 Hz, C*H*-10,14), 7.74 (1H, tt, *J* 7.4, 1.2 Hz, C*H*-12), 7.61 (2H, t, *J* 8.7 Hz, C*H*-11,13), 4.43 (2H, t, *J* 6.3 Hz, CH_2-6), 2.40 (2H, t, *J* 7.3 Hz, CH_2-4), 2.12 (2H, dt, *J* 7.3, 6.3 Hz, CH_2-5) and 1.44 (9H, s, 3 × CH_3-1); **^{13}C NMR** (75 MHz; $CDCl_3$) δ 170.7 (quat., C-3), 158.9 (quat., C-7), 138.0 (quat., C-9), 135.7 (*C*H, C-12), 129.7 (*C*H × 2, C-11,13), 128.5 (*C*H × 2, C-10,14), 110.5 (quat., C-8), 80.8 (quat., C-2), 70.5 (*C*H_2, C-6), 31.3 (CH_2, C-4), 28.1 (*C*H_3 × 3, C-1) and 24.0 (*C*H_2, C-5); ***m/z*** (ES$^+$) 385 ([M+H]$^+$, 100%); **HRMS** *m/z* (ES$^+$) calcd. for $C_{16}H_{21}N_2NaO_7S$ [M+H]$^+$ requires 385.1069, found 385.1072.

4-(3-Carboxypropoxy)-3-(phenylsulfonyl)-1,2,5-oxadiazole 2-oxide, 261



Trifluoroacetic acid (2 mL) was added to a solution of 4-(4-(*tert*-butoxy)-4-oxobutoxy)-3-(phenylsulfonyl)-1,2,5-oxadiazole 2-oxide **265** (200 mg, 0.52 mmol) in dichloromethane (10 mL) and stirred at room temperature overnight. The solvent was removed under reduced pressure to provide 4-(3-carboxypropoxy)-3-(phenylsulfonyl)-1,2,5-oxadiazole 2-oxide **261** (164 mg, 0.50 mmol, 96%) as a white solid, which was used without any further purification: $\boldsymbol{R_f}$ 0.11 (50:50, EtOAc:PE, UV/$KMnO_4$); **m.p.** 129-130 °C, [Lit.[342] 130-131 °C]; **^{1}H NMR** (400 MHz; $CDCl_3$) δ 8.77 (1H, s, *br*, CO_2H), 8.04 (2H, d, *J* 8.3 Hz, C*H*-8,12), 7.75 (1H, tt, *J* 7.5, 1.4 Hz, C*H*-10), 7.61 (2H, t, *J* 8.0 Hz, C*H*-9,11), 4.49 (2H, t, *J* 6.2 Hz, CH_2-4), 2.60 (2H, t, *J* 7.0 Hz, CH_2-2), 2.21 (2H, t, *J* 6.7 Hz, CH_2-3); **^{13}C NMR** (100 MHz; $CDCl_3$) δ 178.4 (quat., C-1″), 158.8 (quat., C-6), 138.0 (quat., C-7), 135.7 (*C*H, C-10), 129.7 (*C*H × 2, C-9,11), 128.5 (*C*H × 2, C-8,12), 110.4 (quat., C-5), 70.2 (CH_2, C-4), 30.1 (CH_2, C-2), 23.6 (CH_2, C-3); ***m/z*** (ES^+) 351 ($[M+Na]^+$, 100%). The data were in agreement with the literature values.[342]

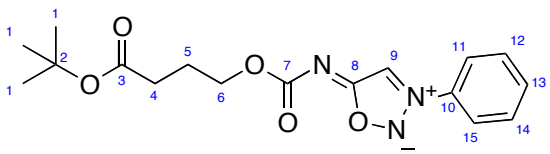
17-(3-Pyridyl)androsta-5,16-dien-3*β*-ol 3-(4-oxo-(phenylsulfonyl)-1,2,5-oxadiazole 2-oxide))butanoate, 253



4-(3-Carboxypropoxy)-3-(phenylsulfonyl)-1,2,5-oxadiazole 2-oxide **261** (53 mg, 0.16 mmol) and DMAP (20 mg, 0.16 mmol) were added to a solution of abiraterone **106** (51 mg, 0.15 mmol) in dichloromethane (7 mL). EDCI.HCl (36 mg, 0.22 mmol) was added in a single

portion and the solution stirred for 36 h. The solution was washed with water (5 mL) and dried over $MgSO_4$. The mixture was filtered and the solvent adsorbed onto silica gel. Purification by silica gel chromatography, eluting with diethyl ether, provided *(((17-(3-pyridyl)androsta*-5,16-*dien-3β-yl)oxy)-4-oxobutoxy)-3-(phenylsulfonyl)*-1,2,*5-oxadiazole 2-oxide* **253** (62 mg, 0.09 mmol, 64%) as a fawn solid: R_f 0.62 (50:50, EtOAc:cyclohexane, UV/cerium phosphomolybdate); **m.p.** 60-61 °C; $[\alpha]_D^{20}$ -13.0, (*c* = 1.0, $CHCl_3$); ν_{max} (thin film)/cm^{-1} 2936, 2855, 1727, 1616, 1522, 1450, 1369, 1260, 1171, 1087, 1023, 999, 799, 734; **^{1}H NMR** (300 MHz; $CDCl_3$) δ 8.60 (1H, d, *J* 1.7 Hz, C*H*-2′), 8.44 (1H, d, *J* 4.5 Hz, C*H*-6′), 8.04 (2H, d, *J* 8.0 Hz, C*H*-8″,12″), 7.76 (1H, tt, *J* 7.4, 1.3 Hz, C*H*-10″), 7.66-7.61 (3H, m, C*H*-4′,9″,11″), 7.21 (1H, dd, *J* 8.0, 5.1 Hz, C*H*-5′), 6.00 (1H, dd, *J* 3.9, 1.7 Hz, C*H*-16), 5.38 (1H, d, *J* 4.9 Hz, C*H*-6), 4.68-4.61 (1H, m, C*H*-3), 4.48 (2H, t, *J* 7.2 Hz, CH_2-4″), 2.50 (2H, t, *J* 7.2 Hz, CH_2-2″), 2.33-2.23 (3H, m, CH_2-1, CH_AH_B-12), 2.20 (2H, pentet, *J* 6.7 Hz, CH_2-3″), 2.09-2.00 (2H, m, CH_AH_B-7, CH_AH_B-12,), 1.88-1.83 (2H, m, CH_AH_B-2, CH_AH_B-4), 1.75 (1H, td, *J* 22.0, 11.0, 5.0 Hz, C*H*-8), 1.69-1.44 (7H, m, CH_AH_B-2, CH_AH_B-7, CH-9, CH_2-11, CH_2-15), 1.16-1.10 (2H, m, CH_AH_B-4, C*H*-14), 1.06 (3H, s, CH_3-19), 1.04 (3H, s, CH_3-18); **^{13}C NMR** (100 MHz; $CDCl_3$) δ 171.6 (quat., C-1″), 158.9 (quat., C-5″), 151.6 (quat., C-17), 147.9 (*C*H, C-2′), 147.8 (*C*H, C-6′), 139.9 (quat., C-5), 138.1 (quat., C-7″), 135.7 (*C*H, C-10″), 133.8 (*C*H, C-4′), 133.0 (quat., C-1′), 129.7 (*C*H × 2, C-9″,11″), 129.3 (*C*H, C-16), 128.6 (*C*H × 2, C-8″,12″), 123.1 (*C*H, C-5), 122.4 (*C*H, C-6), 110.5 (quat., C-6″), 74.4 (*C*H, C-3), 70.4 (CH_2, C-4″), 57.5 (*C*H, C-9), 50.3 (*C*H, C-14), 47.3 (quat., C-13), 38.1 (CH_2, C-1), 36.9 (CH_2, C-4), 36.8 (quat., C-10), 35.2 (CH_2, C-7), 31.8 (CH_2, C-12), 31.5 (CH_2, C-15), 30.4 (*C*H, C-8), 30.4 (CH_2, C-2″), 27.8 (CH_2, C-2), 23.9 (CH_2, C-3″), 20.8 (CH_2, C-11), 19.3 (CH_3, C-19), 16.6 (CH_3, C-18); ***m/z*** (ES^+) 618 ($[M+Na]^+$, 100%); **HRMS** *m/z* (ES^+) calcd. for $C_{36}H_{41}N_3NaO_5$ $[M+Na]^+$ requires 618.2944, found 618.2954; **CHN** Anal. calcd. For $C_{36}H_{41}N_3O_5$: C, 72.58; H, 6.95; N, 7.05. Found C, 72.66; H, 7.01; N, 7.11.

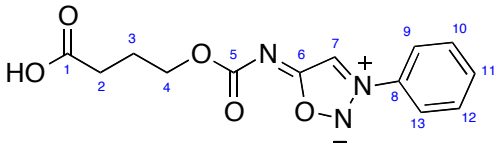
N-((4-(*tert*-Butoxy)-4-oxobutoxy)carbonyl)-3-phenylsydnonimine, 267



Alcohol **262** (500 mg, 3.12 mmol) and *N*-((4-nitrophenoxy)carbonyl)-3-phenylsydnonimine **218** (1.11 g, 3.44) were dissolved in acetonitrile (25 mL) and heated under reflux for 24 h. The solvent was removed under reduced pressure and the residue adsorbed onto silica gel. Purification by silica gel chromatography, eluting with dichloromethane and acetone (100: 0 to

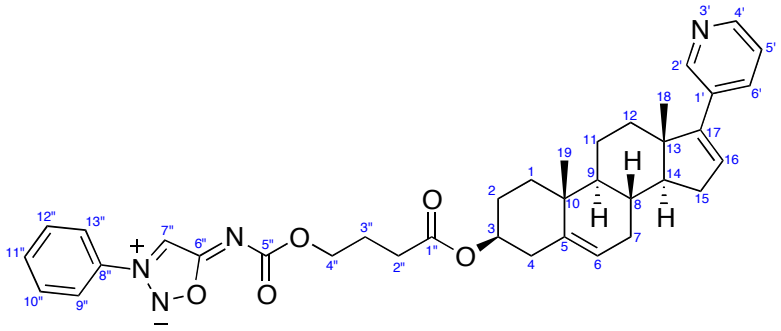
90:10), provided *N-((4-(tert-butoxy)-4-oxobutoxy)carbonyl)-3-phenylsydnonimine* **267** (726 mg, 2.09 mmol, 67%) as an amber solid: $\boldsymbol{R_f}$ 0.37 (90:10, CH_2Cl_2:acetone, UV/cerium phosphomolybdate); **m.p.** 45-47 °C; $\boldsymbol{\nu_{max}}$ (thin film)/cm^{-1} 2977, 1884, 1726, 1631, 1496, 1394, 1368, 1259, 1158, 766; **^{1}H NMR** (300 MHz; $CDCl_3$) δ 8.14 (1H, s C*H*-9), 7.80 (2H, d, *J* 8.4 Hz, C*H*-11,15), 7.75-7.63 (3H, m, C*H*-12,13,14), 4.16 (2H, t, *J* 6.4 Hz, CH_2-6), 2.39 (2H, t, *J* 7.7 Hz, CH_2-4), 1.98 (2H, dt, *J* 7.7, 6.4, CH_2-5), and 1.43 (9H, s, 3 × CH_3-1); **^{13}C NMR** (100 MHz; $CDCl_3$) δ 175.2 (quat., C-8), 172.6 (quat., C-3), 161.7 (quat., C-7), 133.9 (quat., C-10), 133.2 (*C*H, C-13), 130.1 (*C*H × 2, C-11,15), 121.6 (*C*H × 2, C-12,14), 102.8 (*C*H, C-9), 80.3 (quat., C-2), 64.7 (*C*H_2, C-6), 32.1 (*C*H_2, C-4), 28.1 (*C*H_3 × 3, C-1) and 24.5 (*C*H_2, C-5); ***m/z*** (ES^+) 370 ($[M+Na]^+$, 100%); **HRMS** *m/z* (ES^+) calcd. for $C_{17}H_{21}N_3NaO_5$ $[M+Na]^+$ requires 370.1379, found 370.1382.

N-((3-Carboxypropoxy)carbonyl)-3-phenylsydnonimine, 266



Trifluoroacetic acid (800 µL) was added to a solution of *N*-((4-(*tert*-butoxy)-4-oxobutoxy)carbonyl)-3-phenylsydnonimine **267** (53 mg, 0.153 mmol) in CH_2Cl_2 (3 mL) at 0 °C. The solution was allowed to warm to room temperature and stirred for 18 h. The solvent was evaporated under reduced pressure and the residue azeotroped with toluene. The residue was uptaken in dichloromethane and adsorbed onto silica gel. Purification by silica gel chromatography, eluting with dichloromethane and methanol (100: 0 to 97:3), provided *N-((3-carboxypropxy)carbonyl)-3-phenylsydnonimine* **266** (46 mg, 0.153 mmol, quant.): $\boldsymbol{R_f}$ 0.37 (90:10, CH_2Cl_2:acetone, UV/cerium phosphomolybdate); **m.p.** 99-101 °C**;** $\boldsymbol{\nu_{max}}$ (thin film)/cm^{-1} 3479, 2977, 1796, 1725, 1597, 1532, 1458, 1369, 1158, 915, 845; **^{1}H NMR** (300 MHz; $CDCl_3$) δ 8.51 (1H, s C*H*-7), 8.30 (1H, s, *br*, CO_2H), 7.76 (2H, d, *J* 8.2 Hz, C*H*-9,13), 7.75-7.63 (3H, m, C*H*-10,11,12), 4.16 (2H, t, *J* 6.4 Hz, CH_2-4), 2.39 (2H, t, *J* 7.7 Hz, CH_2-2) and 1.98 (2H, dt, *J* 7.7, 6.4 Hz, CH_2-3); **^{13}C NMR** (100 MHz; $CDCl_3$) δ 176.9 (quat., C-6), 168.8 (quat., C-1), 153.6 (quat., C-5), 134.7 (*C*H, C-11), 132.8 (quat., C-8), 131.1 (*C*H × 2, C-9,13), 122.1 (*C*H × 2, C-10,12), 106.3 (*C*H, C-7), 67.1 (*C*H_2, C-4), 31.1 (*C*H_2, C-2), and 24.1 (*C*H_2-3); ***m/z*** (ES^+) 314 ($[M+Na]^+$, 100%); **HRMS** *m/z* (ES^+) calcd. for $C_{13}H_{13}N_3NaO_5$ $[M+Na]^+$ requires 314.0753, found 314.0760.

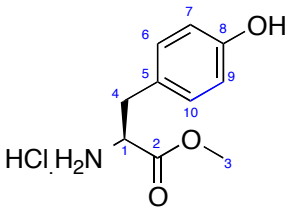
17-(3-Pyridyl)androsta-5,16-dien-3*β*-ol 3-(*N*-((4-oxobutoxy)carbonyl)-3-phenyl-sydnonimine), 254



N-((3-Carboxypropoxy)carbonyl)-3-phenylsydnonimine **266** (46 mg, 0.16 mmol) and DMAP (17 mg, 0.17 mmol) were added to a solution of abiraterone **106** (50 mg, 0.143 mmol) in dichloromethane (7 mL). EDCI.HCl (40 mg, 0.21 mmol) was added in a single portion and the solution stirred for 6 h. The solution was washed with water and dried over $MgSO_4$. The mixture was filtered and the solvent adsorbed onto silica gel. Purification by silica gel chromatography, eluting with ethyl acetate and petroleum ether (15:85 to 25:75), provided *17-(3-pyridyl)androsta*-5,16-*dienyl-3β-(N-((4-oxobutoxy)carbonyl)-3-phenylsydnonimine)* **254** (67 mg, 0.11 mmol, 76%) as an off-white solid: $\boldsymbol{R_f}$ 0.45 (90:10, CH_2Cl_2:acetone, UV/cerium phosphomolybdate); **m.p.** 55-58 °C; $[\boldsymbol{\alpha}]_{\mathbf{D}}^{\mathbf{20}}$-7.4, (*c* = 1.0, $CHCl_3$); $\boldsymbol{\nu_{max}}$ (thin film)/cm^{-1} 3333, 1728, 1651, 1587, 1557, 1509, 1451, 1367, 1214, 1190, 1155, 971, 846; **^{1}H NMR** (300 MHz; $CDCl_3$) δ 8.61 (1H, d, *J* 1.8 Hz, C*H*-2′), 8.45 (1H, dd, *J* 4.9, 1.5 Hz, C*H*-6′), 8.12 (1H, s, CH-7″), 7.82-7.78 (2H, m, CH-9″,13″), 7.72-7.62 (4H, m, C*H*-4′,10″,11″,12″), 7.21 (1H, ddd, *J* 7.9, 4.8, 0.6 Hz, C*H*-5′), 5.99 (1H, dd, *J* 3.2, 1.7 Hz, C*H*-16), 5.41 (1H, d, *J* 5.0 Hz, C*H*-6), 4.68-4.57 (1H, m, C*H*-3), 4.18 (2H, t, *J* 6.2 Hz, CH_2-4″), 2.47 (2H, t, *J* 7.5 Hz, CH_2-2″), 2.39-2.22 (3H, m, CH_2-1, CH_AH$_B$-12), 2.10-1.99 (4H, m, CH_2-3″, CH_AH$_B$-7, CH$_A$*H*$_B$-12), 1.88-1.81 (2H, m, CH_AH$_B$-2, CH_AH$_B$-4), 1.77-1.44 (8H, m, CH$_A$*H*$_B$-2, CH$_A$*H*$_B$-7, CH-8, C*H*-9, CH_2-11, CH_2-15), 1.21-1.10 (2H, m, CH$_A$*H*$_B$-4, C*H*-14), 1.07 (3H, s, CH_3-19) and 1.04 (3H, s, CH_3-18); **^{13}C NMR** (100 MHz; $CDCl_3$) δ 175.3 (quat., C-6″), 172.6 (quat., C-1″), 153.5 (quat., C-5″), 151.7 (quat., C-17), 147.9 (*C*H, C-2′), 147.9 (*C*H, C-6′), 140.0 (quat., C-5), 135.4 (CH, C-11″), 133.9 (*C*H, C-4′), 133.2 (quat., C-8″), 133.0 (quat., C-1′), 130.6 (*C*H × 2, C-9″, C-13″), 129.3 (*C*H, C-16), 123.0 (*C*H, C-5′), 122.2 (*C*H, C-6), 121.6 (*C*H × 2, C-19″, C-12″), 101.4 (CH, C-7″), 73.8 (*C*H, C-3), 64.6 (*C*H_2, C-4″), 57.5 (*C*H, C-9), 50.3 (*C*H, C-14), 47.4 (quat., C-13), 38.2 (*C*H_2, C-1), 36.9 (*C*H_2, C-4), 36.8 (quat., C-10), 35.2 (*C*H_2, C-7), 31.6 (*C*H_2, C-2″), 31.8 (*C*H_2, C-12), 31.5 (*C*H_2, C-15), 30.4 (*C*H, C-8), 27.7 (*C*H_2, C-2), 24.5 (*C*H_2, C-3″), 20.8 (*C*H_2, C-11), 19.3 (*C*H_3, C-19), 16.6 (*C*H_3, C-18); ***m/z*** (ES$^+$) 645 ([M+Na]$^+$, 100%); **HRMS** *m/z* (ES$^+$) calcd. for $C_{37}H_{42}N_4NaO_5$ [M+Na]$^+$ requires 645.3053, found 645.3065; **CHN** Anal. calcd. For $C_{37}H_{42}N_4O_5$: C, 71.36; H, 6.80; N, 9.09. Found C, 71.42; H, 6.82; N, 9.09.

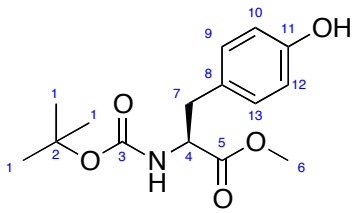
A.6 EXPERIMENTAL PROCEDURES FOR CHAPTER FIVE

(*S*)-Tyrosine methyl ester hydrochloride, 292[370]



Acetyl chloride (15.5 g, 14 mL, 196 mmol) was added dropwise to methanol (110 mL) cooled to 0 °C. Tyrosine **283** (5.4 g, 27.6 mmol) was added and the resulting suspension heated to reflux for 18 h. The resultant solution was cooled to room temperature and the solvent removed under reduced pressure to give a white solid. This was suspended in methanol and the solvent removed under reduced pressure. This procedure was repeated three times to yield (*S*)-tyrosine methyl ester hydrochloride **292** (5.4 g, 27.6 mmol, quant.) as a white solid, which was used without any further purification: **m.p.** 190-193 °C (ethanol), [Lit.[410] 189-190 °C]; $[\alpha]_{\mathbf{D}}^{\mathbf{20}}$+13.0, (*c* = 2, MeOH), [Lit.[411] +12.9 (*c* = 2, MeOH)]; **^{1}H NMR** (300 MHz; d_6-DMSO) δ 8.70 (3H, s, *br*, NH_2.*H*Cl), 6.99 (2H, d, *J* 8.4 Hz, C*H*-7,9), 6.72 (2H, d, *J* 8.4 Hz, C*H*-6,10), 5.08 (1H, s, *br*, O*H*), 4.13-4.07 (1H, m, C*H*-1), 3.62 (3H, s, CH_3-3) and 3.03 (2H, dq, *J* 14.2, 5.2 Hz, CH_2-4); **^{13}C NMR** (100 MHz; d_6-DMSO) δ 169.3 (quat., C-2), 156.6 (quat., C-8), 130.3 (*C*H × 2, C-6,11), 124.3 (quat., C-7), 115.4 (*C*H × 2, C-7,9), 53.5 (*C*H_2, C-4), 52.5 (*C*H_3, C-3) and 35.0 (*C*H, C-1); ***m/z*** (ES$^+$) 196 ([M+H]$^+$, 100%). The data were in agreement with the literature values.[370]

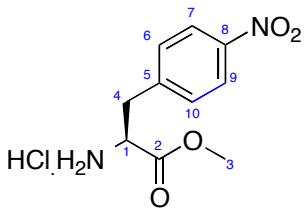
N-tert-Butoxycarbonyl-(*S*)-tyrosine methyl ester, 293[370]



Sodium bicarbonate (21.5 g, 256 mmol) was added to a solution of (*S*)-tyrosine methyl ester hydrochloride **293** (5.0 g, 25.6 mmol) in ethanol (100 mL). Di-tert-butyl dicarbonate (5.6 g, 25.6 mmol) was added and the resulting suspension was stirred for 18 h at room temperature. The suspension was filtered and the solvent removed under reduced pressure to yield *N-tert*-

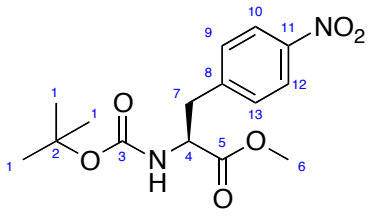
butoxycarbonyl-(*S*)-tyrosine methyl ester **293** (6.00 g, 20.4 mmol, 80%) as a colourless oil that solidified on standing, which was used without any further purification: R_f 0.54 (50:50, EtOAc:PE, UV/cerium phosphomolybdate); **m.p.** 100-103 °C, [Lit.[412] 100-102 °C]; $[\alpha]_D^{20}$ +46.0, (*c* = 1, $CHCl_3$); [Lit.[412] +47.0 (*c* = 1, $CHCl_3$]; **^{1}H NMR** (300 MHz; $CDCl_3$) δ 6.96 (2H, d, *J* 8.4 Hz, C*H*-10,12), 6.73 (2H, d, *J* 8.4 Hz, C*H*-9,13), 5.94 (1H, s, O*H*), 5.95-6.00 (1H, m, N*H*), 4.57-4.50 (1H, m, C*H*-4), 3.71 (3H, s, CH_3-6), 3.06-2.92 (2H, m, CH_2-7) and 1.41 (9H, s, 3 × CH_3-1); **^{13}C NMR** (100 MHz; $CDCl_3$) δ 172.6 (quat., C-5), 155.3 (quat., C-3), 150.1 (quat., C-11), 130.4 (*C*H × 2, C-9,13), 121.3 (quat., C-8), 115.5 (*C*H × 2, C-10,12), 80.1 (quat., C-2), 54.6 (CH_2, C-7), 52.2 (CH_3, C-6), 37.5 (*C*H, C-4) and 28.3 (CH_3 × 3, C-1); ***m/z*** (ES^+) 318 ($[M+Na]^+$, 100%). The data were in agreement with the literature values.[370]

(*S*)-4-Nitrophenylalanine methyl ester, 295[371]



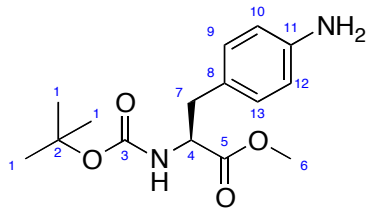
Thionyl chloride (7.5 g, 4.6 mL, 62.8 mmol) was added dropwise to methanol (50 mL) at 0 °C. (*S*)-4-Nitrophenylalanine **294** (6.2 g, 27.2 mmol) was added portionwise and the resulting solution was allowed to warm to room temperature and stirred for 18 h. The solvent and excess thionyl chloride was removed under reduced pressure to give a yellow residue. This was evaporated from methanol (5 × 100 mL) to give (*S*)-4-nitrophenylalanine methyl ester hydrochloride **295** (7.1 g, 27.2 mmol, quant.) as a pale yellow solid, which was used without any further purification: **m.p.** 209-212 °C, [Lit.[413] 221-224 °C]; $[\alpha]_D^{20}$+32.0, (*c* = 1.0, EtOH), [Lit.[413] +32.2, (*c* = 1.0, EtOH)]; **^{1}H NMR** (500 MHz; d_6-DMSO) δ 9.01 (3H, s, *br*, NH_2.*H*Cl), 8.16 (2H, d, *J* 8.7 Hz, C*H*-7,9), 7.59 (2H, d, *J* 8.7 Hz, C*H*-6,10), 4.34 (1H, dd, *J* 7.6, 5.7 Hz, C*H*-1), 3.67 (3H, s, CH_3-3), 3.42 (1H, dd, *J* 14.0, 5.6 Hz, CH_AH_B-4) and 3.30 (1H, dd, *J* 13.8, 7.8 Hz, CH_AH_B-4); **^{13}C NMR** (75 MHz, d_6-DMSO) δ 168.9 (quat., C-2), 146.7 (quat., C-5), 143.0 (quat., C-8), 130.9 (*C*H × 2, C-6,10), 123.5 (*C*H × 2, C-7,9), 52.3 (*C*H, C-1) and 52.6 (CH_3, C-3); ***m/z*** (ES^+) 225 ($[M+H]^+$, 100%). The data were in agreement with the literature values.[371]

4-Nitro-*N*-*tert*-butoxycarbonyl-(*S*)-phenylalanine methyl ester, 296[371]



Triethylamine (2.90 g, 4.0 mL, 28.7 mmol) and di-*tert* butyl dicarbonate (3.10, 14.1 mmol) was added to a suspension of (*S*)-methyl 4-nitrophenylalanine hydrochloride **295** (3.5 g, 13.4 mmol) in dichloromethane (25 mL). The resulting suspension was stirred at room temperature for 18 h. The reaction mixture was diluted with water (50 mL), and the organic layer separated. The aqueous layer was extracted with dichloromethane (50 mL). The organic extracts were combined and washed with brine (50 mL), dried over $MgSO_4$. The mixture was filtered and the solvent removed under reduced pressure to give 4-nitro-*N*-*tert*-butoxycarbonyl-(*S*)-phenylalanine methyl ester **296** (4.35 g, 13.4 mmol, quant.) as a white solid, which was used without any further purification: $\boldsymbol{R_f}$ 0.72 (30:70, EtOAc:PE, UV/cerium phosphomolybdate); **m.p.** 99-100 °C, [Lit.[412] 99-101 °C]; $[\alpha]_D^{20}$+52.9, (*c* = 1, $CHCl_3$); [Lit.[412] +53.0, (*c* = 1, $CHCl_3$)]; **^{1}H NMR** (500 MHz; $CDCl_3$) δ 8.13 (2H, d, *J* 8.7 Hz, C*H*-10,12), 7.29 (2H, d, *J* 8.7 Hz, C*H*-9,13), 5.09 (1H, d, *J* 7.5, N*H*), 4.63-4.57 (1H, m, C*H*-4), 3.71 (3H, s, CH_3-6), 3.25 (1H, dd, *J* 13.7, 5.7 Hz, CH_AH_B-7), 3.07 (1H, dd, *J* 14.0, 7.2 Hz, CH_AH_B-7) and 1.37 (9H, s, 3 × CH_3-1); **^{13}C NMR** (100 MHz; $CDCl_3$) δ 171.6 (quat., C-5), 154.9 (quat., C-3), 147.2 (quat., C-11), 144.0 (quat., C-8), 130.6 (*C*H × 2, C-9,13), 123.7 (*C*H × 2, C-10,12), 80.4 (quat., C-2), 54.1 (CH_2, C-7), 52.6 (CH_3, C-6), 45.8 (*C*H, C-4) and 28.3 (CH_3 × 3, C-1); ***m/z*** (ES$^+$) 347 ([M+Na]$^+$, 100%). The data were in agreement with the literature values.[371]

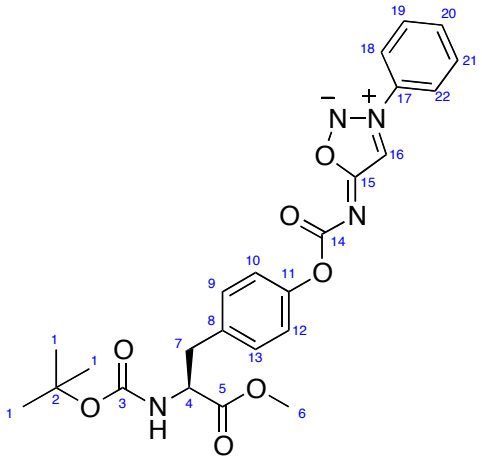
4-Amino-*N*-tert-butoxycarbonyl-(*S*)-phenylalanine methyl ester, 297[371]



10% Pd/C (15% in weight, 150 mg) was added to a solution of 4-nitro-*N*-tert-butoxycarbonyl-(*S*)-phenylalanine methyl ester **296** (1.0 g, 3.09 mmol) and ammonium formate (1.27 g, 20.1 mmol) in methanol (20 mL) and the suspension stirred for 3 h. The suspension was filtered

through Celite and the solvent evaporated. The residue was uptaken in ethyl acetate (50 mL) and washed with water (3 × 20 mL). The organic layer was dried over $MgSO_4$, filtered and the solvent removed under reduced pressure to yield 4-amino-*N*-tert-butoxycarbonyl-(*S*)-phenylalanine methyl ester **297** (0.91 g, 3.09 mmol, quant.) as a yellow solid, which was used without any further purification: $\boldsymbol{R_f}$ 0.41 (30:70, EtOAc: PE, UV/cerium phosphomolybdate); **m.p.** 85-90 °C, [Lit.[414] 85-87 °C]; $[\boldsymbol{\alpha}]_{\mathbf{D}}^{\mathbf{20}}$ +42.1, (*c* = 1, $CHCl_3$); [Lit.[412] +42.0, (*c* = 1.0 Hz, $CHCl_3$)]; **^{1}H NMR** (500 MHz; $CDCl_3$) δ 6.89 (2H, d, *J* 8.0 Hz, C*H*-9,13), 6.61 (2H, d, *J* 8.0 Hz, C*H*-10,12), 4.96 (1H, d, *J* 8.3, N*H*), 4.52-4.48 (1H, m, C*H*-4), 3.70 (3H, s, CH_3-6), 3.00-2.92 (2H, m, CH_2-7) and 1.41 (9H, s, 3 × CH_3-1); **^{13}C NMR** (100 MHz; $CDCl_3$) δ 172.6 (quat., C-5), 155.2 (quat., C-3), 145.3 (quat., C-11), 130.1 (*C*H × 2, C-9,13), 125.7 (quat., C-8), 115.3 (*C*H × 2, C-10,12), 79.8 (quat., C-2), 54.6 (CH_2, C-7), 52.2 (CH_3, C-6), 37.4 (*C*H, C-4) and 28.3 (CH_3 × 3, C-1); ***m/z*** (ES^+) 317 ($[M+Na]^+$, 100%). The data were in agreement with the literature values.[371]

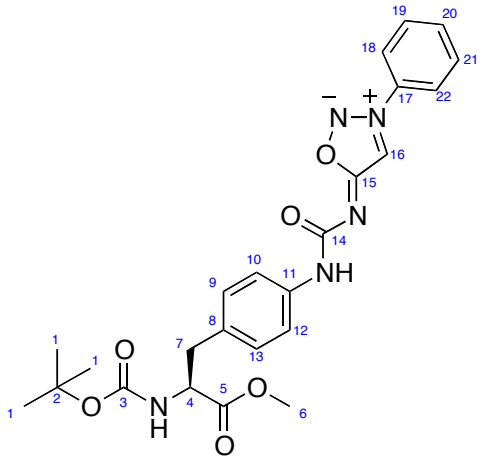
(*S*)-*N*-((4-(2-((*tert*-Butoxycarbonyl)amino)-3-methoxy-3-oxopropyl)phenyloxy)-carbonyl)-3-phenylsydnonimine, 298



N-*tert*-Butoxycarbonyl-(*S*)-tyrosine methyl ester **293** (532 mg, 1.80 mmol) and *N*-((4-nitrophenoxy)carbonyl)-3-phenylsydnonimine **218** (587 mg, 1.80 mmol) were dissolved in acetonitrile (15 mL) and heated under reflux for 24 h. The solvent was removed under reduced pressure and the residue uptaken in dichloromethane (10 mL) and adsorbed onto silica gel. Silica gel chromatography, eluting with CH_2Cl_2 and acetone (100: 0 to 90:10) provided ((*S*)-*N*-((*4*-(*2*-((*tert-butoxycarbonyl)amino)-3-methoxy-3-oxopropyl)phenyloxy) carbonyl)-3-phenylsydnonimine* **298** (772 mg, 1.60 mmol, 89%) as a pale yellow crystalline solid: $\boldsymbol{R_f}$ 0.30 (50:50, EtOAc:PE, UV/cerium phosphomolybdate); **m.p.** 58-60 °C; $[\boldsymbol{\alpha}]_{\mathbf{D}}^{\mathbf{20}}$ +31.0, (*c* = 1.0, $CHCl_3$); $\boldsymbol{\nu_{max}}$ (thin film)/cm^{-1} 1744, 1678, 1586, 1471, 1367, 1282, 1213, 971, 1018; **^{1}H NMR** (500 MHz; $CDCl_3$) δ 8.17 (1H, s, 1H, C*H*-16), 7.79 (2H, d, *J* 8.0 Hz, C*H*-18,22), 7.71 (1H, t, *J*

7.4 Hz, C*H*-20), 7.65 (2H, t, *J* 8.0 Hz, C*H*-19,21), 7.13-7.09 (4H, m, C*H*-9, 10, 12, 13), 5.02 (1H, d, *J* 8.4, N*H*-Boc), 4.55 (1H, dd, *J* 13.7, 6.0 Hz, C*H*-4), 3.69 (3H, s, CH_3-6), 3.08 (1H, dd, *J* 14.0, 5.7 Hz, CH_AH_B-7), 3.03 (1H, dd, *J* 13.8, 6.1 Hz, CH_AH_B-7) and 1.40 (9H, s, 3 × CH_3-1); **^{13}C NMR** (125 MHz; $CDCl_3$) δ 175.7 (quat., C-15), 172.4 (quat., C-5), 160.4 (quat., C-14), 155.2 (quat., C-3), 151.1 (quat., C-11), 133.7 (quat., C-17), 133.3 (*C*H, C-20), 132.7 (quat., C-8), 130.7 (*C*H × 2, C-9,13), 130.0 (*C*H × 2, C-18,22), 121.9 (*C*H × 2, C-10,12), 121.5 (*C*H × 2, C-19,21), 103.4 (*C*H, C-16), 80.0 (quat., C-2), 54.4 (*C*H, C-4), 52.3 (CH_3, C-6), 37.6 (CH_2, C-7) and 28.3 (CH_3 × 3, C-1); ***m/z*** (ES^+) 505 ($[M+Na]^+$, 100%); **HRMS** *m/z* (ES^+) calcd. for $C_{24}H_{26}N_4NaO_7$ $[M+Na]^+$ requires 505.1699, found 505.1672.

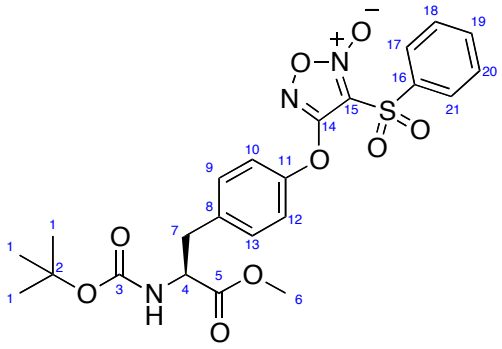
(*S*)-*N*-((4-(2-((*tert*-Butoxycarbonyl)amino)-3-methoxy-3-oxopropyl)phenyl)-carbamoyl)-3-phenylsydnonimine, 299



4-Amino-*N*-tert-butoxycarbonyl-(*S*)-phenylalanine methyl ester **297** (186 mg, 0.63 mmol) and *N*-((4-nitrophenoxy)carbonyl)-3-phenylsydnonimine **218** (206 mg, 0.63 mmol) were dissolved in acetonitrile (15 mL) and heated under reflux for 24 h. The solvent was removed under reduced pressure and the residue uptaken in dichloromethane (10 mL) and adsorbed onto silica gel. Silica gel chromatography, eluting with CH_2Cl_2 and acetone (100: 0 to 90:10) provided (*S*)-*N*-((*4*-(*2*-((*tert-butoxycarbonyl)amino)-3-methoxy-3-oxopropyl)phenyl)-carbamoyl)-3-phenylsydnonimine* **299** (243 mg, 0.51 mmol, 88%) as a yellow solid: ***R*$_f$** 0.19 (50:50, EtOAc:PE, UV/cerium phosphomolybdate); **m.p.** 62-64 °C; **$[\alpha]_D^{20}$** +30.3, (*c* = 1.0 , $CHCl_3$); **ν_{max}** (thin film)/cm^{-1} 1740, 1708, 1650, 1591, 1517, 1411, 1365, 1244, 1166; **^{1}H NMR** (500 MHz; $CDCl_3$) δ 8.25 (1H, s, C*H*-16), 7.80-7.77 (2H, m, C*H*-18,22), 7.71-7.60 (3H, m, C*H*-19, 20, 21), 7.45 (2H d, *J* 8.5 Hz, C*H*-10,12), 7.28 (1H, s, N*H*), 7.05-7.00 (2H, m, C*H*-9,13), 5.10-5.01 (1H, m, N*H*Boc), 4.53 (1H, dd, *J* 13.3, 5.8 Hz, C*H*-4), 3.69 (3H, s, CH_3-6), 3.08-2.94 (CH_2-7) and 1.40 (9H, s, 3 × CH_3-1); **^{13}C NMR** (100 MHz; $CDCl_3$) δ 173.4 (quat., C-15), 172.5 (quat., C-5), 159.4 (quat., C-14), 155.2 (quat., C-3), 138.5 (quat., C-8), 134.0 (quat., C-17),

133.0 (*C*H, C-20), 130.5 (*C*H × 2, C-18,22), 130.1 (quat., C-11), 129.7 (*C*H × 2, C-9,13), 121.5 (*C*H × 2, C-20, 21), 119.0 (*C*H × 2, C-10,12), 103.4 (*C*H, C-16), 80.1 (quat., C-2), 54.5 (CH_2, C-7), 52.2 (CH_3, C-6), 37.7 (*C*H, C-4) and 28.3 (CH_3 × 3, C-1); ***m/z*** (ES^+) 504 ($[M+Na]^+$, 100%); **HRMS** *m/z* (ES^+) calcd. for $C_{24}H_{27}N_5NaO_6$ $[M+Na]^+$ requires 504.1859, found 504.1861.

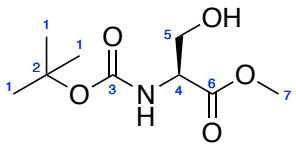
(*S*)-4-(4-(2-((*tert*-Butoxycarbonyl)amino)-3-methoxy-3-oxopropyl)phenoxy)-3-(phenylsulfonyl)-1,2,5-oxadiazole 2-oxide, 300



DBU (544 µL, 3.64 mmol) was added to a solution of *N*-*tert*-butoxycarbonyl-(*S*)-tyrosine methyl ester **293** (537 mg, 1.82 mmol) in dichloromethane (10 mL) and the solution stirred vigorously. *Bis*(phenylsulfonyl)-1,2,5-oxadiazole 2-oxide **53** (666 mg, 1.82 mmol) was added and stirring continued for 2 h. The reaction mixture was washed with distilled water (20 mL) and with HCl (2 N, 2 × 15 mL) and with brine (20 mL), the organic layer was dried over Na_2SO_4. The mixture was filtered and the solvent removed under reduced pressure to give the crude product. This product was purified by silica gel chromatography, eluting with dichloromethane and hexane (50:50 to 100:0) to give (*S*)-*4*-(*4*-(*2*-((*tert-butoxycarbonyl)amino)-3-methoxy-3-oxopropyl)phenoxy)-*3*-(*phenylsulfonyl*)-1,2,*5-oxadiazole 2-oxide* **300** (595 mg, 1.71 mmol, 86%) as a white solid; $\boldsymbol{R_f}$ 0.54 (30:70, EtOAc:PE, UV/cerium phosphomolybdate); **m.p.** 129-131 °C; $[\alpha]_D^{20}$ +29.2, (*c* = 1, $CHCl_3$); ν_{max} (thin film)/cm^{-1} 1746, 1713, 1619, 1535, 1449, 1366, 1275, 1200, 1167, 1019; **^{1}H NMR** (500 MHz; $CDCl_3$) δ 8.06 (2H, d, *J* 8.2 Hz, C*H*-17,21), 7.76 (1H, tt, *J* 7.5, 1.2 Hz, C*H*-19), 7.62 (2H, t, *J* 8.2 Hz, C*H*-18,20), 7.22-7.18 (4H, m, C*H*-9,10,12,13), 5.09 (1H, d, *J* 8.1, N*H*-Boc), 4.57 (1H, dd, *J* 13.9, 6.5 Hz, C*H*-4), 3.69 (3H, s, CH_3-6), 3.14 (1H, dd, *J* 13.9, 5.8 Hz, CH_AH_B-7), 3.03 (1H, dd, *J* 13.9, 6.3 Hz, CH_AH_B-7) and 1.39 (9H, s, 3 × *C*H_3-1); **^{13}C NMR** (125 MHz; $CDCl_3$) δ 172.1 (quat., C-5), 158.3 (quat., C-14), 155.0 (quat., C-3), 151.6 (quat., C-11), 137.8 (quat., C-16), 135.9 (*C*H, C-19), 135.0 (quat., C-8), 130.9 (*C*H × 2, C-9,13), 129.8 (*C*H × 2, C-17,21), 128.6 (*C*H × 2, C-18,20), 119.9 (*C*H × 2, C-10,12), 110.7 (quat., C-14), 80.1 (quat., C-2), 54.4 (*C*H, C-4), 52.4 (*C*H_3, C-6), 37.7 (*C*H_2, C-

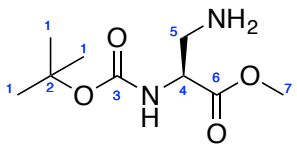
7) and 28.3 (CH_3 × 3, C-1); ***m/z*** (ES^+) 542 ($[M+Na]^+$, 100%); **HRMS** *m/z* (ES^+) calcd. for $C_{23}H_{25}N_3NaO_9S$ $[M+Na]^+$ requires 542.1209, found 542.1210.

N-(*tert*-Butoxycarbonyl)-L-serine methyl ester, 301[373]



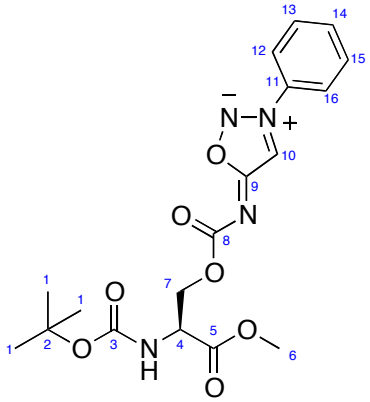
Thionyl chloride 11.9 g, 7.3 mL, 100 mmol) was added dropwise to a suspension of L-serine **285** (10.0 g, 95 mmol) in methanol (150 mL) at 0 °C following the addition the solution was allowed to warm to room temperature and stirred overnight. The solvent was removed under reduced pressure, and the resulting solid was suspended in methanol and the solvent removed reduced pressure; this procedure was repeated a further two times. The solid was then suspended in diethyl ether, filtered and washed with diethyl ether and dried under vacuum to provide L-serine methyl ester **303** (11.3 g). L-serine methyl ester **303** (11.3 g) was suspended in acetonitrile (200 mL) and di-*tert*-butyl-dicarbonate (21.8 g, 100 mmol) in acetonitrile (100 mL) followed by triethylamine (19.3 g, 26.6 mL, 190 mmol) in acetonitrile (100 mL) in one portion and the resultant solution stirred at room temperature overnight. CH_2Cl_2 (1000 mL) was added and organics extracted with aq. HCl (1 N, 500 mL) and saturated sodium bicarbonate (100 mL). The organic layer was washed with brine (200 mL), dried over $MgSO_4$, filtered and the solvent removed under reduced pressure to furnish *N*-(tert-butoxycarbonyl)-L-serine methyl ester **301** (21.8 g, 95 mmol, quant) as a viscous colourless oil, which was used without any further purification: $\boldsymbol{R_f}$ 0.35 (80:20, EtOAc:PE); $\boldsymbol{[\alpha]_D^{20}}$ +9.0, (*c* = 1.0 , $CHCl_3$); [Lit.[373] +9.0, (*c* = 1.0 , $CHCl_3$]; **^{1}H NMR** (300 MHz; $CDCl_3$) δ 5.52 (1H, d, *J* 7.0, *br*, N*H*-Boc), 4.37 (1H, s *br*, C*H*-4), 4.01-3.81 (2H, m, CH_2-5), 3.76 (3H, s, CH_3-7), 2.78 (1H, t, *J* 5.9, *br*, O*H*) and 1.43 (9H, s, 3 × CH_3); **^{13}C NMR** (100 MHz; $CDCl_3$) δ 171.6 (quat.), 156.0 (quat.), 80.6 (quat.), 63.7 (CH_2), 56.0 (*C*H), 52.9 (CH_3) and 28.5 (CH_3 × 3); ***m/z*** (ES^+) 242 ($[M+Na]^+$, 100%). The data were in agreement with the literature values.[373]

(*S*)-Methyl 3-amino-2-(*tert*-butoxycarbonylamino)propanoate, 302[374]



TMS-diazomethane solution (2.0 M in ether, 7.9 mL, 15.8 mmol) was added dropwise to a solution of (*S*)-2-((*tert*-butoxycarbonyl)amino)-3-aminopropionic acid **304** (3.0 g, 14.7 mmol) in dichloromethane (50 mL) and methanol (5 mL). The mixture was stirred at room temperature for 3 h, following this time a pale yellow solution had formed. The solvent was removed under reduced pressure to yield methyl 3-amino-2-(*tert*-butoxycarbonylamino)propanoate **302** (3.21 g, 14.7 mmol, quant.) as a colourless oil, which was used without any further purification: $\boldsymbol{R_f}$ 0.35 (90:10, CH_2Cl_2:MeOH, UV/cerium phosphomolybdate); $[\alpha]_D^{20}$ -25.1, (*c* = 1, MeOH); [Lit.[415] -25.0, (*c* = 1, MeOH]; **^{1}H NMR** (500 MHz, $CDCl_3$) δ 5.44 (1H, d, *J* 5.3, N*H*-Boc), 4.32-4.27 (1H, m, C*H*-4), 3.75 (3H, s, CH_3-7), 3.03 (2H, d, *J* 4.8 Hz, CH_2-5), 1.43 (9H, s, 3 × CH_3-1) and 1.40-1.35 (2H, m, NH_2); **^{13}C NMR** (125 MHz, $CDCl_3$) δ 172.1 (quat., C-6), 155.5 (quat., C-3), 80.0 (quat., C-2), 55.9 (*C*H, C-4), 52.4 (CH_3, C-7), 43.9 (CH_2, C-5) and 28.3 (CH_3 × 3, C-1); ***m/z*** (ES^+) 241 ($[M+Na]^+$, 100%); ***m/z*** (ES^+) 587 ($[M+Na]^+$, 100%). The data were in agreement with the literature values.[374]

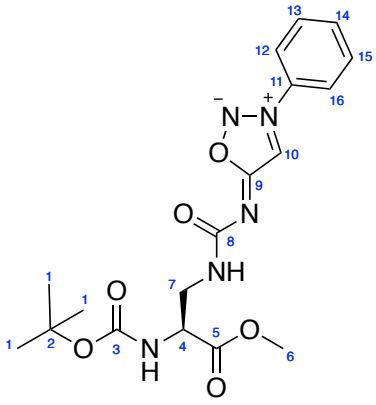
(*S*)-*N*-((2-((*tert*-Butoxycarbonyl)amino)-3-methoxy-3-oxopropoxy)carbonyl)-3-phenylsydnonimine, 305



N-(tert-Butoxycarbonyl)-L-serine methyl ester **301** (250 mg, 1.14 mmol) and *N*-((4-nitrophenoxy)carbonyl)-3-phenylsydnonimine **218** (372 mg, 1.14 mmol) were dissolved in acetonitrile (15 mL) and heated under reflux for 24 h. The solvent was removed under reduced pressure and the residue uptaken in dichloromethane (10 mL) and adsorbed onto silica gel. Silica gel chromatography, eluting with CH_2Cl_2 and acetone (100: 0 to 90:10), provided (*S*)-*N*-

(((*2*-((*tert-butoxycarbonyl)amino)-3-methoxy-3-oxopropoxy)carbonyl)-3-phenylsydnonimine **305** (272 mg, 0.67 mmol, 59%) as a colourless crystalline solid: R_f 0.24 (90:10, CH_2Cl_2:MeOH, UV/cerium phosphomolybdate); **m.p.** 40-41 °C; $[\alpha]_D^{20}$ +17.9, (*c* = 1.0, $CHCl_3$); ν_{max} (thin film)/cm^{-1} 2978, 1793, 1712, 1585, 1390, 1377, 1282, 1069, 972; **^{1}H NMR** (300 MHz; $CDCl_3$) δ 8.12 (1H, s C*H*-10), 7.81 (2H, d, *J* 8.2 Hz, C*H*-12,16), 7.75 -7.69 (3H, m, C*H*-13,14,15), 5.52 (1H, d, *J* 8.4, N*H*-Boc), 4.61-4.55 (2H, m, CH_2-7), 4.41-4.35 (1H, m, C*H*-4), 3.77 (3H, s, CH_3-6) and 1.44 (9H, s, 3 × CH_3); **^{13}C NMR** (100 MHz; $CDCl_3$) δ 175.1 (quat), 170.5 (quat.), 160.1 (quat), 155.3 (quat.), 133.6 (quat), 133.1 (*C*H), 130.5 (*C*H × 2), 121.4 (*C*H × 2), 103.0 (*C*H), 89.8 (quat.), 65.2 (*C*H_2), 53.1 (*C*H), 52.5 (*C*H_3) and 28.1 (*C*H_3 × 3); ***m/z*** (ES^+) 429 ($[M+Na]^+$, 100%); **HRMS** *m/z* (ES^+) calcd. for $C_{18}H_{22}N_4NaO_7$ $[M+Na]^+$ requires 429.1386, found 429.1390.

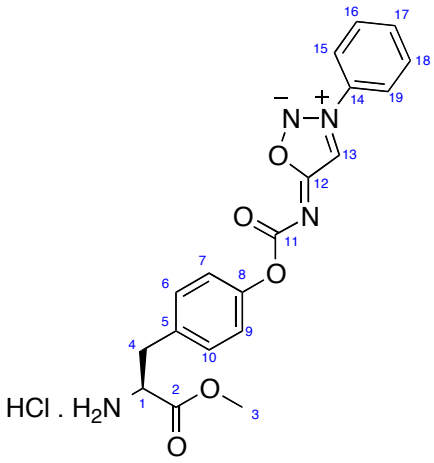
(*S*)-*N*-((2-((*tert*-Butoxycarbonyl)amino)-3-methoxy-3-oxopropoxy)carbamoyl)-3-phenylsydnonimine, 306



(*S*)-Methyl 3-amino-2-(*tert*-butoxycarbonylamino)propanoate **302** (250 mg, 1.14 mmol) and *N*-((4-nitrophenoxy)carbonyl)-3-phenylsydnonimine **218** (372 mg, 1.14 mmol) were dissolved in acetonitrile (15 mL) and heated under reflux for 24 h. The solvent was removed under reduced pressure and the residue uptaken in dichloromethane (10 mL) and adsorbed onto silica gel. Silica gel chromatography, eluting with CH_2Cl_2 and acetone (100: 0 to 90:10), provided (*S*)-*N*-((*2*-((*tert-butoxycarbonyl)amino)-3-methoxy-3-oxopropoxy)carbamoyl)-3-phenylsydnonimine **306** (254 mg, 0.627 mmol, 55%) as a yellow crystalline solid: R_f 0.13 (95:5, CH_2Cl_2:acetone, UV/cerium phosphomolybdate); **m.p.** 150 °C (decomp.); $[\alpha]_D^{20}$ +18.1, (*c* = 1.0 , $CHCl_3$); ν_{max} (NaCl/thin film)/cm^{-1} 1760, 1600, 1477, 1450, 1325, 1145, 1032, 980; **^{1}H NMR** (500 MHz, $CDCl_3$) δ 8.16 (1H, s, C*H*-10), 7.79 (2H, d, *J* 7.8 Hz, C*H*-12,16), 7.69 (1H, t, *J* 7.3 Hz, C*H*-14), 7.63 (2H, t, *J* 7.8 Hz, C*H*-13,15), 7.37 (1H, s, *br*, N*H*-urea), 5.87 (1H, t, *J* 7.2, N*H*-Boc), 4.41-4.38 (1H, m, C*H*-4), 3.76-3.63 (5H, m, CH_3-6, CH_2-7) and 1.41 (9H, s, 3 × *C*H_3-1); **^{13}C NMR** (125 MHz, $CDCl_3$) δ 172.9 (quat., C-9), 171.6 (quat., C-5), 162.1 (quat., 8), 155. (quat., C-3),

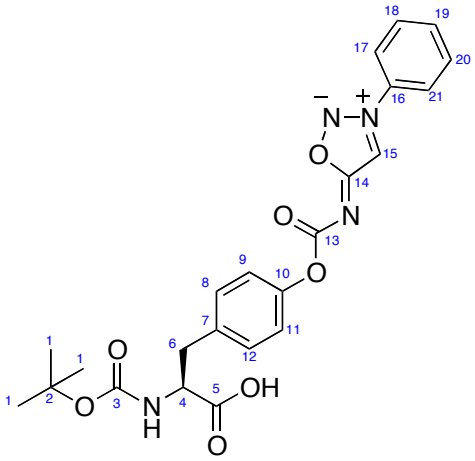
134.0 (quat., C-14), 132.9 (*C*H, C-14), 130.5 (*C*H × 2, C-13,15), 121.6 (*C*H × 2, C-12,16), 102.4 (*C*H, C-10), 79.9 (quat., C-2), 54.8 (*C*H, C-4), 52.5 (CH_3, C-6), 42.2 (CH_2, C-7) and 28.3 (CH_3 × 3, C-1); ***m/z*** (ES^+) 428 ($[M+Na]^+$, 100%); **HRMS** *m/z* (ES^+) calcd. for $C_{18}H_{23}N_5NaO_6$ $[M+Na]^+$ requires 428.1546, found 428.1550.

(*S*)-*N*-((4-(2-Amino-3-methoxy-3-oxopropyl)phenoxy)carbonyl)-3-phenylsydnonimine hydrochloride, 307



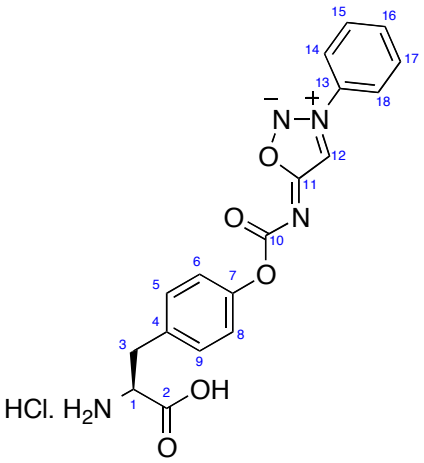
HCl in dioxane (4M, 2 mL, 8.0 mmol) was added to a solution of (*S*)-*N*-((4-(2-((*tert*-butoxycarbonyl)amino)-3-methoxy-3-oxopropyl)phenyloxy)carbonyl)-3-phenylsydnonimine **298** (92 mg, 0.19 mmol) in dichloromethane (5 mL) cooled to 0 °C. The solution was stirred at 0 °C for 3 h and the solvent evaporated under reduced pressure to yield a white residue. Trituration with diethyl ether provided (*S*)-*N*-((*4-(2-amino-3-methoxy-3-oxopropyl)phenoxy)carbonyl)-3-phenylsydnonimine hydrochloride* **307** (78 mg, 0.19 mmol, quant.) as a white solid; **m.p.** 117-119 °C; $[\alpha]_D^{20}$+2.6, (*c* = 1.0, MeOH); ν_{max} (thin film)/cm^{-1} 1780, 1734, 1515, 1313, 1247, 1227, 1068, 868; **^{1}H NMR** (500 MHz; $CDCl_3$) δ 8.82-8.75 (4H, m, C*H*-13, NH_2.*H*Cl), 8.09 (2H, d, *J* 7.6 Hz, C*H*-15,19), 7.83-7.71 (3H, m, C*H*-16,17, 18), 7.28 (2H d, *J* 8.4 Hz, C*H*-6,10), 7.13 (2H d, *J* 8.4 Hz, C*H*-7,9), 4.29-4.23 (1H, m, C*H*-1), 3.68 (3H, s, CH_3-3), 3.22 (1H, dd, *J* 14.2, 5.7 Hz, CH_AH$_B$-4), and 3.12 (1H, dd, *J* 14.2, 7.1 Hz, CH$_A$$H_B$-4); **^{13}C NMR** (75 MHz; d_6-DMSO) δ 173.7 (quat., C-12), 169.3 (quat., C-2), 157.4 (quat., C-11), 150.9 (quat., C-8), 133.4 (quat., C-5), 133.2 (*C*H, C-17), 131.3 (quat., C-14), 130.3 (*C*H × 4, C-6,10,15,19), 122.6 (*C*H × 2, C-16,18), 121.8 (*C*H × 2, C-7,9), 105.4 (*C*H, C-13), 53.1 (*C*H, C-1), 52.6 (CH_3, C-3) and 35.1 (CH_2, C-4); ***m/z*** (ES^+) 383 ($[M+H]^+$, 100%); **HRMS** *m/z* (ES^+) calcd. for $C_{19}H_{19}N_4O_5$ $[M+H]^+$ requires 383.1355, found 383.1360.

(*S*)-*N*-((4-(2-((tert-Butoxycarbonyl)amino)-2-carboxyethyl)phenoxy)carbonyl) -3-phenylsydnonimine, 308



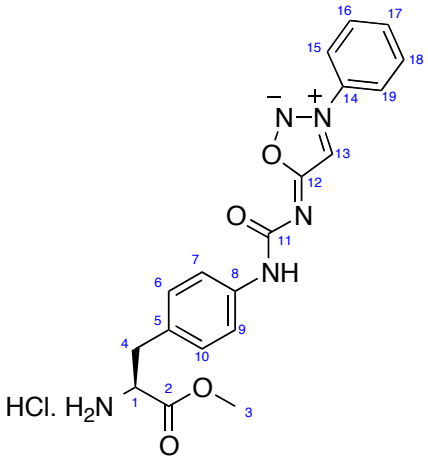
Sodium hydroxide (17 mg, 0.42 mmol) was added to a solution of (*S*)-*N*-((4-(2-((*tert*-butoxycarbonyl)amino)-3-methoxy-3-oxopropyl)phenyloxy)carbonyl)-3-phenylsydnonimine **298** (100 mg, 0.21 mmol) in THF (2 mL) and water (2 mL). The resulting solution was stirred for 30 min and cooled to 0 °C. The solution was acidified (pH = 2) with aq. hydrochloric acid (2 N) with continued stirring. The cooled solution was extracted with ethyl acetate (3 × 20 mL), and the combined organic extracts were washed with brine (20 mL), dried over Na_2SO_4. The mixture was filtered and the solvent removed under reduced pressure to yield (*S*)-*5*-((*4*-(*2*-((*tert-butoxycarbonyl)amino)-2-carboxyethyl)phenoxy)carbonyl)-3-phenylsydnonimine* **308** (92 mg, 0.20 mmol, 95%) as a white solid, which was used without any further purification: $\boldsymbol{R_f}$ 0.63 (100:1, EtOAc:AcOH, UV/cerium phosphomolybdate); **m.p.** 115-118 °C; $[\boldsymbol{\alpha}]_{\mathbf{D}}^{\mathbf{20}}$ -24.8, (*c* = 0.1 , MeOH); $\boldsymbol{\nu_{max}}$ (thin film)/cm^{-1} 1727, 1623, 1580, 1502, 1469, 1365, 1295, 1250, 1187, 1166; **^{1}H NMR** (500 MHz; $CDCl_3$) δ 8.62 (1H, s, C*H*-15), 8.06 (2H, d, *J* 7.6 Hz, C*H*-17,21), 7.80-7.70 (3H, m, C*H*-18, 19, 20), 7.20 (2H d, *J* 8.3 Hz, C*H*-8, 12), 7.10 (2H d, *J* 8.4 Hz, C*H*-9, 11), 6.51 (1H, s, *br*, N*H*), 4.01-3.96 (1H, m, C*H*-4), 3.06 (1H, dd, *J* 14.5, 4.9 Hz, CH_AH_B-6), 2.88 (1H, dd, *J* 13.4, 8.1 Hz, CH_AH_B-4) and 1.34 (9H, s, 3 × CH_3-1); **^{13}C NMR** (75 MHz; d_6-DMSO) δ 174.9 (quat., C-15), 174.4 (quat., C-5), 158.0 (quat., C-13), 155.1 (quat., C-3), 150.3 (quat., C-10), 135.0 (quat., C-7). 133.6 (quat., C-16), 133.0 (*C*H, C-19), 130.2 (*C*H × 2, C-17,21), 129.9 (*C*H × 2, C-8, 12), 122.4 (*C*H × 2, C-18,20), 121.2 (*C*H × 2, C-9, 11), 104.7 (*C*H, C-15), 77.7 (quat., C-2), 55.8 (*C*H, C-4), 36.2 (CH_2, C-6) and 28.2 (CH_3 × 3, C-1); ***m/z*** (ES^+) 491 ($[M+Na]^+$, 100%); **HRMS** *m/z* (ES^+) calcd. for $C_{23}H_{24}N_4NaO_5$ $[M+Na]^+$ requires 491.1543, found 491.1540.

(*S*)-*N*-((4-(2-Amino-2-carboxyethyl)phenyl)carbonyl)-3-phenylsydnonimine hydrochloride, 287



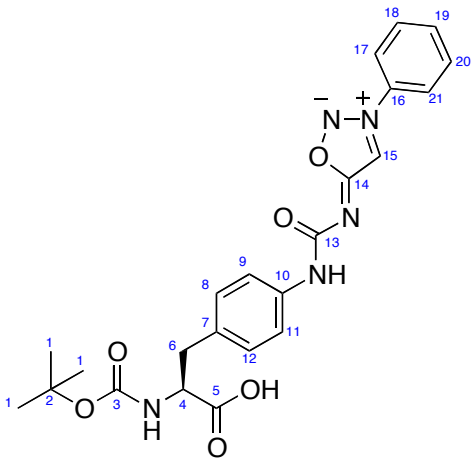
Sodium hydroxide (17 g, 0.42 mmol) was added to a solution of (*S*)-*N*-((4-(2-((*tert*-butoxycarbonyl)amino)-3-methoxy-3-oxopropyl)phenyloxy)carbonyl)-3-phenylsydnonimine **298** (100 mg, 0.21 mmol) in THF (2 mL) and water (2 mL). The resulting solution was stirred for 30 min and cooled to 0 °C. The solution was acidified (pH = 2) with aq. hydrochloric acid (2 N) with continued stirring. The cooled solution was extracted with ethyl acetate (3 × 20 mL), and the combined organic extracts were evaporated at reduced pressure. The residue was uptaken in dichloromethane (4 mL) and cooled to 0 °C. HCl in dioxane (4M, 1.5 mL, 6 mmol) was added dropwise and the solution stirred at 0 °C for 1 h. Following this time the solvent was evaporated under reduced pressure and the residue triturated with diethyl ether to give (*S*)-*N*-((*4-(2-amino-2-carboxyethyl)phenyl)carbonyl)-3-phenylsydnonimine hydrochloride* **287** (65 mg, 0.160 mmol, 77%) as a white powder: **m.p**. 108-111 °C; $[\alpha]_D^{20}$ -0.6, (*c* = 1.0 , MeOH); ν_{max} (thin film)/cm^{-1} 3036, 1755, 1629, 1597, 1544, 1507, 1368, 1256, 1216, 1193; **^{1}H NMR** (500 MHz; $CDCl_3$) δ 8.73 (1H, s, C*H*-12), 8.51 (3H, s, *br*, NH_2.*H*Cl), 8.03 (2H, d, *J* 8.0 Hz, C*H*-14,18), 7.79 (1H, tt, *J* 7.5, 2.3 Hz, C*H*-16), 7.74 (2H, t, *J* 8.1 Hz, C*H*-15,17), 7.31 (2H d, *J* 8.5 Hz, C*H*-5,9), 7.12 (2H d, *J* 8.5 Hz, C*H*-6,8), 4.16 (1H, dd, *J* 11.6, 5.9 Hz, C*H*-1) and 3.15 (2H, d, *J* 6.2 Hz, CH_2-3); **^{13}C NMR** (125 MHz; d_6-DMSO) δ 174.6 (quat., C-11), 170.8 (quat., C-2), 158.3 (quat., C-10), 151.4 (quat., C-7), 133.9 (quat., C-13), 133.7 (*C*H, C-16), 132.0 (quat., C-4), 130.8 (*C*H × 2, C-14,18), 130.8 (*C*H × 2, C-5,9), 123.1 (*C*H × 2, C-15,17), 122.3 (*C*H × 2, C-6,8), 105.8 (*C*H, C-15), 55.4 (*C*H, C-1) and 35.5 (*C*H$_2$, C-3); ***m/z*** (ES$^+$) 391 ([M+Na]$^+$, 100%); **HRMS** *m/z* (ES$^+$) calcd. for $C_{18}H_{16}N_4NaO_5$ [M+Na]$^+$ requires 391.1018, found 391.1022.

(*S*)-*N*-((4-(2-Amino-3-methoxy-3-oxopropyl)phenyl)carbamoyl)-3-phenylsydnonimine hydrochloride, 309



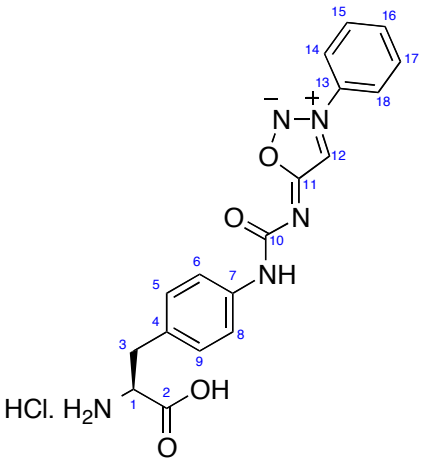
HCl in dioxane (4M, 2 mL, 8.0 mmol) was added to a solution of (*S*)-*N*-((4-(2-((*tert*-butoxycarbonyl)amino)-3-methoxy-3-oxopropyl)phenyl)carbamoyl)-3-phenylsydnonimine **299** (100 mg, 0.21 mmol) in dichloromethane (5 mL) cooled to 0 °C. The solution was stirred at 0 °C for 3 h and the solvent evaporated under reduced pressure to yield a yellow residue. Trituration with diethyl ether provided (*S*)-*N*-((*4-(2-amino-3-methoxy-3-oxopropyl)phenyl)carbamoyl)-3-phenylsydnonimine hydrochloride* **309** (87 mg, 0.21 mmol, quant.) as a orange solid, which was used without any further purification: **m.p.** 160 °C (decomp.); $[\alpha]_D^{20}$ +5.2, (*c* = 0.8, MeOH); ν_{max} (thin film)/cm^{-1} 3334, 1738, 1706, 1650, 1591, 1517, 1365, 1274, 1246, 1165; **^{1}H NMR** (500 MHz; $CDCl_3$) δ 9.61 (1H, s, N*H*), 9.11 (1H, s, C*H*-13), 8.65 (3H, s, *br*, NH_2.*H*Cl), 8.13 (2H, m, C*H*-15,19), 7.86-7.74 (3H, m, C*H*-16,17,18), 7.54 (2H d, *J* 8.3 Hz, C*H*-7,9), 7.18 (2H, d, *J* C*H*-6,10), 4.25-4.19 (1H, m, C*H*-1), 3.68 (3H, s, CH_3-3) and 3.18-3.00 (2H, m, CH_2-4); **^{13}C NMR** (75 MHz; d_6-DMSO) δ 176.0 (quat., C-12), 169.4 (quat., C-2), 159.5 (quat., C-11), 139.2 (quat., C-8), 133.5 (quat., C-5), 133.4 (quat., C-17), 133.1 (*C*H, C-14), 130.4 (*C*H × 2, C-15,19), 127.2 (*C*H × 2, C-6,10), 122.6 (*C*H × 2, C-16,18), 118.5 (*C*H × 2, C-7,9), 106.2 (*C*H, C-16), 53.2 (CH_2, C-1), 52.6 (CH_3, C-3) and 35.2 (*C*H, C-4); ***m/z*** (ES$^+$) 382 ([M+H]$^+$, 100%); **HRMS** *m/z* (ES$^+$) calcd. for $C_{19}H_{20}N_5O_4$ [M+H]$^+$ requires 382.1515, found 382.1520.

(*S*)-*N*-((4-(2-((*tert*-Butoxycarbonyl)amino)-2-carboxyethyl)phenoxy)carbamoyl) -*3*-phenylsydnonimine, 310



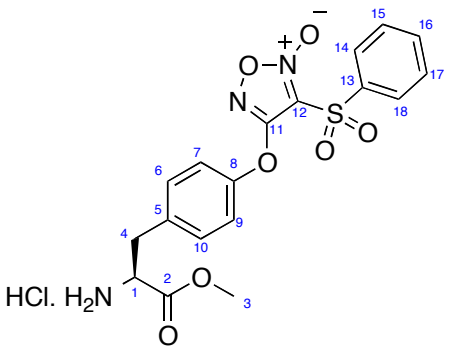
Sodium hydroxide (17 mg, 0.42 mmol) was added to a solution of (*S*)-*N*-((4-(2-((*tert*-butoxycarbonyl)amino)-3-methoxy-3-oxopropyl)phenyl)carbamoyl)-3-phenylsydnonimine **299** (100 mg, 0.21 mmol) in THF (2 mL) and water (2 mL). The resulting solution was stirred for 30 min and cooled to 0 °C. The solution was acidified (pH = 2) with aq. hydrochloric acid (2 N) with continued stirring. The cooled solution was extracted with ethyl acetate (3 × 20 mL), and the combined organic extracts were washed with brine (20 mL), dried over Na_2SO_4. The mixture was filtered and the solvent removed under reduced pressure to yield (*S*)-*N*-((*4*-(*2*-((*tert-butoxycarbonyl)amino)-2-carboxyethyl)phenoxy)carbamoyl)-3-phenylsydnonimine* **310** (85 mg, 0.18 mmol, 88%) as a yellow solid, which was used without any further purification: $\boldsymbol{R_f}$ 0.19 (80:20, CH_2Cl_2:acetone, UV/cerium phosphomolybdate); **m.p.** 151-154 °C; $\boldsymbol{[\alpha]_D^{20}}$ +8.0, (*c* = 1, MeOH); $\boldsymbol{\nu_{max}}$ (thin film)/cm^{-1} 1710, 1650, 1590, 1517, 1411, 1365, 1313, 1245, 1167, 963; **^{1}H NMR** (500 MHz; $CDCl_3$) δ 9.46 (1H, s, NH), 8.55 (1H, s, C*H*-15), 7.86 (2H, d, *J* 7.9 Hz, C*H*-17,21), 7.72 (1H, t, *J* 7.3 Hz, C*H*-19), 7.66 (2H, t, *J* 7.6 Hz, C*H*-18,20), 7.36 (2H, d, *J* 8.0 Hz, C*H*-9, 11), 7.07 (2H, d, *J* 8.1 Hz, C*H*-8, 12), 5.16 (1H, d, *J* 7.7, NH), 4.78 (1H, dd, *J* 13.4, 6.2 Hz, C*H*-4), 3.15-3.05 (2H, m, CH_2-6) and 1.45 (9H, s, 3 × CH_3-1); **^{13}C NMR** (125 MHz; $CDCl_3$) δ 176.8 (quat., C-14), 175.9 (quat., C-5), 155.8 (quat., C-13), 155.3 (quat., C-3), 138.0 (quat., C-7), 133.5 (quat., C-16), 133.3 (*C*H, C-19), 130.6 (*C*H × 2, C-17,21), 130.4 (quat., C-10), 129.9 (*C*H × 2, C-8, 12), 121.7 (*C*H × 2, C-18,20), 119.0 (*C*H × 2, C-9, 11), 80.0 (quat., C-2), 54.8 (*C*H, C-4), 37.7 (CH_2, C-6) and 28.4 (CH_3 × 3, C-1); ***m/z*** (ES^+) 490 ($[M+H]^+$, 100%); **HRMS** *m/z* (ES^+) calcd. for $C_{23}H_{25}N_5NaO_6$ $[M+H]^+$ requires 490.1703, found 490.1711.

(*S*)-*N*-((4-(2-Amino-2-carboxyethyl)phenyl)carbamoyl)-3-phenylsydnonimine hydrochloride, 288



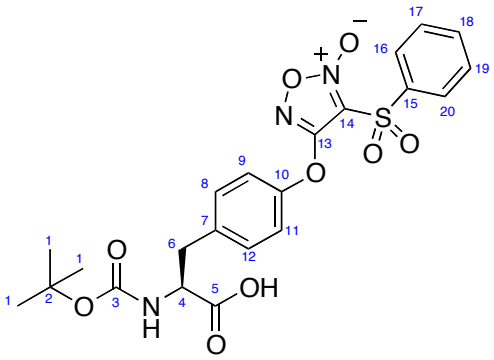
Sodium hydroxide (17 mg, 0.42 mmol) was added to a solution of (*S*)-*N*-((4-(2-((*tert*-butoxycarbonyl)amino)-3-methoxy-3-oxopropyl)phenyl)carbamoyl)-3-phenylsydnonimine **299** (100 mg, 0.21 mmol) in THF (2 mL) and water (2 mL). The resulting solution was stirred for 30 min and cooled to 0 °C. The solution was acidified (pH = 2) with aq. hydrochloric acid (2 N) with continued stirring. The cooled solution was extracted with ethyl acetate (3 × 20 mL), and the combined organic extracts were evaporated at reduced pressure. The residue was uptaken in dichloromethane (4 mL) and cooled to 0 °C. HCl in dioxane (4M, 1.5 mL, 6 mmol) was added dropwise and the solution stirred at 0 °C for 1 h. Following this time the solvent was evaporated under reduced pressure and the residue triturated with diethyl ether to give (*S*)-*N*-((*4*-(*2-amino-2-carboxyethyl)phenyl)carbamoyl)-3-phenylsydnonimine* **288** (63 mg, 0.16 mmol, 75%) as a yellow solid: **m.p.** 200 °C (decomp); $[\alpha]_D^{20}$ -1.6, (*c* = 1.0 , MeOH); ν_{max} (thin film)/cm^{-1} 1700, 1648, 1520, 1409, 1360, 1240, 970; **^{1}H NMR** (300 MHz; $CDCl_3$) δ 9.59 (1H, s, NH), 9.11 (1H, s, C*H*-12), 8.42 (3H, s, *br*, NH_2.*H*Cl), 8.13 (2H, m, C*H*-14,18), 7.83 (1H, tt, *J* 7.5, 1.5 Hz, C*H*-16), 7.76 (2H, d, *J* 7.8 Hz, C*H*-15,17), 7.54 (2H d, *J* 8.5 Hz, C*H*-6,8), 7.16 (2H, d, *J* 8.5 Hz, C*H*-5,9), 4.10-4.07 (1H, m, C*H*-1) and 3.11-3.06 (2H, m, CH_2-3); **^{13}C NMR** (75 MHz; d_6-DMSO) δ 174.8 (quat., C-11), 170.3 (quat., C-2), 152.7 (quat., C-10), 139.0 (quat., C-7), 134.1 (*C*H, C-16), 133.5 (quat., C-13), 130.4 (quat., C-4), 130.0 (*C*H × 2, C-14,18), 129.8 (*C*H × 2, C-6,8), 123.3 (*C*H × 2, C-15,17), 117.8 (*C*H × 2, C-5,9), 106.2 (*C*H, C-12), 53.2 (*C*H_2, C-1) and 35.8 (*C*H, C-4); ***m/z*** (ES$^+$) 390 ([M+H]$^+$, 100%); **HRMS** *m/z* (ES$^+$) calcd. for $C_{23}H_{25}N_5NaO_6$ [M+H]$^+$ requires 390.1178, found 490.1711.

(*S*)-4-(4-(2-Amino-3-methoxy-3-oxopropyl)phenoxy)-3-(phenylsulfonyl)-1,2,5-oxadiazole 2-oxide hydrochloride, 311



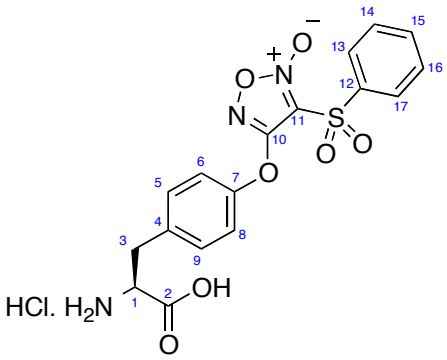
HCl in dioxane (4M, 2 mL, 8.0 mmol) was added to a solution of (*S*)-4-(4-(2-((tert-butoxycarbonyl)amino)-3-methoxy-3-oxopropyl)phenoxy)-3-(phenylsulfonyl)-1,2,5-oxadiazole 2-oxide **300** (100 mg, 0.19) in dichloromethane (5 mL) cooled to 0 °C. The solution was stirred at 0 °C for 3 h and the solvent evaporated under reduced pressure to yield a white residue. Trituration with diethyl ether provided (*S*)-*4*-(*4*-(*2-amino-3-methoxy-3-oxopropyl)phenoxy)-3-*(*phenylsulfonyl)*-1,2,*5-oxadiazole 2-oxide hydrochloride* **311** (87 mg, 0.19 mmol, quant.) as a white solid: **m.p.** 174-177 °C; $[\alpha]_D^{20}$ +9.3, (*c* = 0.9, MeOH); ν_{max} (thin film)/cm^{-1} 1742, 1617, 1503, 1357, 1275, 1275, 1161, 1017, 990**; ^{1}H NMR** (300 MHz; d_6-DMSO) δ 8.72 (3H, s, *br*, NH_2.*H*Cl), 8.05 (2H, d, *J* 8.1 Hz, C*H*-14,18), 7.92 (1H, tt, *J* 7.5, *J* 1.4 Hz, C*H*-16), 7.77 (2H, t, *J* 7.9 Hz, C*H*-15,17), 7.41-7.35 (4H, m, C*H*-6,7,9,10), 4.31 (1H, t, *J* 6.1 Hz, C*H*-1), 3.68 (3H, s, CH_3-3), 3.23 (1H, dd, *J* 6.1, *J* 14.2 Hz, CH_AH$_B$-4) and 3.14 (1H, dd, *J* 7.3, *J* 14.2 Hz, CH$_A$$H_B$-4); **^{13}C NMR** (75 MHz; d_6-DMSO) δ 169.3 (quat., C-2), 158.4 (quat., C-11), 151.8 (quat., C-8), 136.9 (quat., C-5), 136.3 (*C*H, C-16), 133.1 (quat., C-13), 131.1 (*C*H × 2, C-6,10), 130.1 (*C*H × 2, C-14, C-18), 128.5 (*C*H × 2, C-15,17), 119.8 (*C*H × 2, C-7,10), 111.2 (quat., C-12), 53.0 (*C*H, C-1), 52.6 (*C*H$_3$, C-3) and 35.0 (*C*H$_2$, C-4); ***m/z*** (ES$^+$) 420 ([M+H]$^+$, 100%); **HRMS** *m/z* (ES$^+$) calcd. for $C_{18}H_{18}N_3O_7S$ [M+H]$^+$ requires 420.0865, found 420.0870.

(*S*)-4-(4-(2-((*tert*-Butoxycarbonyl)amino)-2-carboxyethyl)phenoxy)-3-(phenylsulfonyl)-1,2,5-oxadiazole 2-oxide, 312



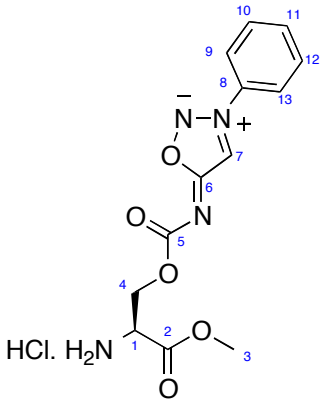
Sodium hydroxide (15 mg, 0.39 mmol) was added to a solution of (*S*)-4-(4-(2-((*tert*-butoxycarbonyl)amino)-3-methoxy-3-oxopropyl)phenoxy)-3-(phenylsulfonyl)-1,2,5-oxadiazole 2-oxide **300** (100 mg, 0.19 mmol) in THF (2 mL) and water (2 mL). The resulting solution was stirred for 30 min and cooled to 0 °C. The solution was acidified (pH = 2) with aq. hydrochloric acid (2 N) with continued stirring. The cooled solution was extracted with ethyl acetate (3 × 20 mL), and the combined organic extracts were washed with brine (20 mL), dried over Na2SO4. The mixture was filtered and the solvent removed under reduced pressure to yield (*S*)-*4*-(*4*-(*2*-((*tert-butoxycarbonyl)amino)-2-carboxyethyl)phenoxy)-3-(phenylsulfonyl)*-1,2,5-oxadiazole 2-*oxide **312** (92 mg, 0.20 mmol, 90%) as a white solid, which was used without any further purification: $\boldsymbol{R_f}$ 0.48 (100:1, EtOAc:AcOH, UV/cerium phosphomolybdate); **m.p.** 66-68 °C; $\boldsymbol{[\alpha]_D^{20}}$ +6.4, (*c* = 1.0 , MeOH); $\boldsymbol{\nu_{max}}$ (thin film)/cm^{-1} 1717, 1619, 1535, 1505, 1445, 1368, 1267, 1167, 1019; **^{1}H NMR** (500 MHz; $CDCl_3$) δ 8.10 (2H, d, *J* 8.4 Hz, C*H*-16,20), 7.89 (1H, tt, *J* 6.7, *J* 1.2 Hz, C*H*-18), 7.64 (2H, t, *J* 7.7 Hz, C*H*-17,19), 7.29-7.25 (4H m, C*H*-8,9,11,12), 5.02 (1H, d, *J* 8.7, N*H*-Boc), 4.62 (1H, dd, *J* 11.9, 6.3 Hz, C*H*-4), 3.24 (1H, dd, *J* 14.3, 5.6 Hz, CH_AH$_B$-6), 3.09 (1H, dd, *J* 14.3, 6.6 Hz, CH$_A$$H_B$-4) and 1.42 (9H, s, 3 × CH_3-1); **^{13}C NMR** (75 MHz; d_6-DMSO) δ 175.5 (quat., C-5), 158.4 (quat., C-13), 155.4 (quat., C-3), 151.6 (quat., C-10), 137.9 (quat., C-7), 135.9 (*C*H, C-18), 134.7 (quat., C-15), 131.0 (*C*H × 2, C-8,12), 129.8 (*C*H × 2, C-16, 20), 128.7 (*C*H × 2, C-17,19), 120.0 (*C*H × 2, C-9,11), 110.7 (quat., C-14), 80.6 (quat., C-2), 54.2 (*C*H, C-4), 37.2 (CH_2, C-6) and 28.3 (CH_3 × 3, C-1); ***m/z*** (ES$^+$) 528 ([M+Na]$^+$, 100%); **HRMS** *m/z* (ES$^+$) calcd. for $C_{22}H_{23}N_3NaO_9S$ [M+Na]$^+$ requires 528.1053, found 528.2050.

(*S*)-4-(4-(Amino-2-carboxyethyl)phenoxy)-3-(phenylsulfonyl)-1,2,5-oxadiazole 2-oxide, 289



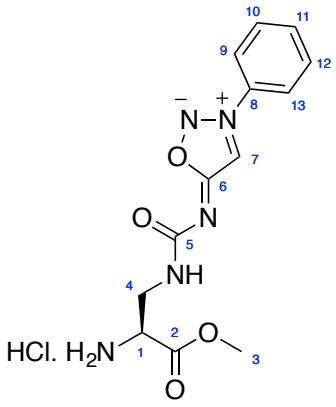
Sodium hydroxide (15 mg, 0.39 mmol) was added to a solution of (*S*)-4-(4-(2-((*tert*-butoxycarbonyl)amino)-3-methoxy-3-oxopropyl)phenoxy)-3-(phenylsulfonyl)-1,2,5-oxadiazole 2-oxide **300** (100 mg, 0.19 mmol) in THF (2 mL) and water (2 mL). The resulting solution was stirred for 30 min and cooled to 0 °C. The solution was acidified (pH = 2) with aq. hydrochloric acid (2 N) with continued stirring. The cooled solution was extracted with ethyl acetate (3 × 20 mL), and the combined organic extracts were evaporated at reduced pressure. The residue was uptaken in dichloromethane (4 mL) and cooled to 0 °C. HCl in dioxane (4M, 1.5 mL, 6 mmol) was added dropwise and the solution stirred at 0 °C for 1 h. Following this time the solvent was evaporated under reduced pressure and the residue triturated with diethyl ether to give (*S*)-*4*-(*4*-(*2-amino-2-carboxyethyl)phenoxy)-3-*(*phenylsulfonyl)*-1,2,*5-oxadiazole 2*-oxide **289** (68 mg, 0.15 mmol, 80%) as a white powder: **m.p.** 250 °C (decomp); $[\alpha]_D^{20}$ -0.52, (*c* = 0.7 , MeOH); ν_{max} (thin film)/cm^{-1} 1727, 1623, 1534, 1449, 1352, 1256, 1201, 1163, 1083; **^{1}H NMR** (300 MHz; d_6-DMSO) δ 8.57 (3H, s, *br*, NH_2.*H*Cl), 8.05 (2H, d, *J* 8.4 Hz, C*H*-13,17), 7.92 (1H, tt, *J* 7.5, *J* 2.2 Hz, C*H*-15), 7.78 (2H, t, *J* 8.0 Hz, C*H*-14,16), 7.42-7.36 (4H, m, C*H*-5,6,8,9), 4.08 (1H, t, *J* 7.2 Hz, C*H*-1) and 3.21 (2H, d, *J* 7.2 Hz, CH_2-3); **^{13}C NMR** (75 MHz; d_6-DMSO) δ 174.6 (quat., C-2), 158.4 (quat., C-10), 152.1 (quat., C-7), 137.2 (quat., C-4), 135.4 (*C*H, C-15), 134.1 (quat., C-13), 131.7 (*C*H × 2, C-5,9), 130.6 (*C*H × 2, C-14,18), 129.0 (*C*H × 2, C-14,16), 120.0 (*C*H × 2, C-6,8), 108.8 (quat., C-11), 53.8 (*C*H., C-1) and 35.3 (*C*H$_2$, C-3); ***m/z*** (ES$^+$) 406 ([M+H]$^+$, 100%); **HRMS** *m/z* (ES$^+$) calcd. for $C_{17}H_{16}N_3O_7S$ [M+H]$^+$ requires 406.0709, found 406.0713.

(*S*)-*N*-((2-Amino-3-methoxy-3-oxopropoxy)carbonyl)-3-phenylsydnonimine hydrochloride, 313



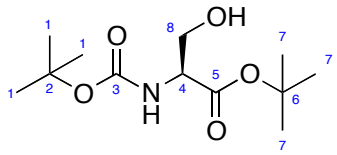
HCl in dioxane (4M, 2 mL, 8.0 mmol) was added to a solution of (*S*)-*N*-(((2-((*tert*-butoxycarbonyl)amino)-3-methoxy-3-oxopropoxy)carbonyl)-3-phenylsydnonimine **305** (100 mg mg, 0.225 mmol) in dichloromethane (5 mL) cooled to 0 °C. The solution was stirred at 0 °C for 3 h and the solvent evaporated under reduced pressure to yield a tan residue. Trituration with diethyl ether provided (*S*)-*N*-(((*2-amino-3-methoxy-3-oxopropoxy)carbonyl)-3-phenylsydnonimine hydrochloride* **313** (84 mg, 0.25 mmol, quant.) as an off-white solid: **m.p.** 102-104 °C; $[\alpha]_D^{20}$ +9.4, (*c* = 0.9, MeOH); ν_{max} (thin film)/cm^{-1} 1682, 1583, 1519, 1471, 1366, 1313, 1213 1187, 969; **^{1}H NMR** (300 MHz; d_6-DMSO) δ 8.95 (3H, s, *br*, NH_2.*H*Cl), 8.78 (1H, s *CH*-7), 8.12-8.04 (2H, m, C*H*-9,13), 7.83-7.71 (3H, m, C*H*-10,11,12), 4.54-4.40 (3H, m, C*H*-1, CH_2-4) and 3.78 (3H, s, CH_3-3); **^{13}C NMR** (100 MHz; d_6-DMSO) δ 173.1 (quat., C-6), 167.6 (quat., C-1), 157.5 (quat., C-5), 133.3 (*C*H, C-11), 130.3 (*C*H × 2, C-10,12), 122.5 (*C*H × 2, C-9,13), 105.4 (*C*H, C-7), 62.8 (*C*H$_2$, C-4), 53.1 (*C*H$_3$, C-3) and 51.5 (*C*H, C-1); ***m/z*** (ES$^+$) 307 ([M+Na]$^+$, 100%); **HRMS** *m/z* (ES$^+$) calcd. for $C_{13}H_{15}N_4O_5$ [M+Na]$^+$ requires 307.1042, found 307.1049.

(*S*)-*N*-(((2-Amino-3-methoxy-3-oxopropoxy)carbonyl)-3-phenylsydnonimine hydrochloride, 314



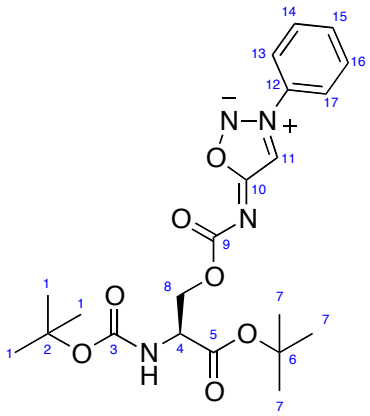
HCl in dioxane (4M, 2 mL, 8.0 mmol) was added to a solution of (*S*)-*N*-(((2-((*tert*-butoxycarbonyl)amino)-3-methoxy-3-oxopropoxy)carbamoyl)-3-phenylsydnonimine **306** (100 mg, 0.25 mmol) in dichloromethane (5 mL) cooled to 0 °C. The solution was stirred at 0 °C for 3 h and the solvent evaporated under reduced pressure to yield a tan residue. Trituration with diethyl ether provided (*S*)-*N*-(((*2-amino-3-methoxy-3-oxopropoxy)carbamoyl)-3-phenylsydnonimine hydrochloride* **314** (84 mg, 0.25 mmol, quant.) as an off-white solid: **m.p**. 150-152; $[\alpha]_D^{20}$ +11.0, (*c* = 1.0, MeOH); ν_{max} (NaCl/thin film)/cm^{-1} 1744, 1609, 1546, 1514, 1495, 1416, 1315, 1237, 1050, 823; **^{1}H NMR** (500 MHz, d_6-DMSO) δ 9.25 (1H, s, C*H*-7), 8.89-8.79 (3H, s, *br*, NH_2.*H*Cl), 8.14 (2H, d, *J* 7.7 Hz, C*H*-9,13), 7.84-7.77 (3H, m, C*H*-10, 11, 12), 4.14-4.09 (1H, s, *br*, C*H*-1) and 3.75-3.71 (5H, m, CH_2-4, CH_3-3); **^{13}C NMR** (125 MHz, d_6-DMSO) δ 176.5 (quat., C-6), 168.8 (quat., C-2), 167.7 (quat., C-5), 134.3 (*C*H, C-11), 133.3 (quat., C-8), 130.9 (*C*H × 2, C-9,13), 123.4 (*C*H × 2, C-10,12), 107.5 (*C*H, C-7), 53.0 (*C*H$_3$, C-3), 52.2 (*C*H, C-1) and 39.9 (*C*H$_2$, C-4); ***m/z*** (ES$^+$) 306 ([M+Na]$^+$, 100%); **HRMS** *m/z* (ES$^+$) calcd. for $C_{13}H_{16}N_5O_6$ [M+H]$^+$ requires 306.1202, found 306.1220.

N-(*tert*-Butoxycarbonyl)-L-serine *tert*-butyl ester, 317



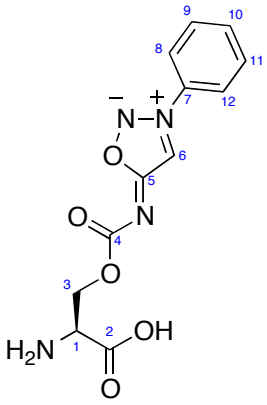
Aqueous sodium hydroxide (1 N, 19.5 mL) was added to a solution of L-serine **285** (1.00 g, 9.5 mmol) in dioxane cooled to 0 °C. Di-*tert*-butyl dicarbonate (2.47 g, 1.13 mmol) was added portionwise and the resultant solution stirred overnight at room temperature. Aqueous citric acid (10%, 50 mL) was added slowly and the solution extracted with ethyl acetate (3 × 100 mL). The organic extracts were combined and washed with brine (100 mL), dried over $MgSO_4$, filtered and the solvent removed under reduced pressure to furnish *N*-(*tert*-butoxycarbonyl)-L-serine (1.94 g) as a colourless oil. *N,N*-Dimethylformamide di-*tert*-butyl acetal (DMF-DBA, 5.27 g, 6.2 mL, 26.9 mmol) was added to a solution of *N*-(*tert*-butoxycarbonyl)-L-serine (1.00 g, 4.89 mmol) in toluene (8 mL). The solution was heated to reflux for 18 h. Aqueous sodium bicarbonate (5% of saturated, 20 mL) was added and the mixture stirred for 30 min. Methanol was added to the mixture until a homogenous layer was obtained. The solution was extracted with ethyl acetate (50 mL). The organic layer was washed with water (3 × 10 mL) and brine (20 mL), dried over $MgSO_4$, filtered and the solvent was removed under reduced pressure and the residue uptaken in dichloromethane and adsorbed onto silica gel. Silica gel chromatography, eluting with ethyl acetate and hexanes (50:50), furnished *N*-(*tert*-butoxycarbonyl)-L-serine *tert*-butyl ester **317** (830 mg, 3.18 mmol, 65%, over two steps) as a colourless oil which solidified on standing: $\boldsymbol{R_f}$ 0.34 (50:50, EtOAc: PE, $KMnO_4$); **m.p.** 79-82 °C [Lit.,[416] 79.0-83.5°C]; $[\boldsymbol{\alpha}]_{\boldsymbol{D}}^{\boldsymbol{20}}$ -25.0, (*c* = 2, EtOH, [Lit.,[417] -25.0, (*c* = 2, EtOH)]; **^{1}H NMR** (400 MHz; $CDCl_3$) δ 5.43 (1H, s, *br*, NH), 4.27-4.20 (1H, m, C*H*-4), 3.88 (2H, d, *J* 3.8 Hz, CH_2-8), 2.25 (1H, s, OH), 1.47 (9H, s, 3 × CH_3-7) and 1.43 (9H, s, 3 × CH_3-1); **^{13}C NMR** (75 MHz; $CDCl_3$) δ 169.8 (quat., C-5), 155.9 (quat., C-3), 82.7 (quat., C-6), 80.2 (quat., C-2), 64.1 (CH_2, C-8), 53.4 (*C*H, C-4), 28.3 (9H, s, 3 × CH_3-1) and 28.0 (9H, s, 3 × CH_3-7); ***m/z*** (ES^+) 262 ($[M+H]^+$, 100%). The data were in agreement with the literature values.[416]

(*S*)-*N*-((3-(*tert*-Butoxy)-2-((*tert*-butoxycarbonyl)amino)-3-oxopropoxy)carbonyl)-3-phenylsydnonimine, 318



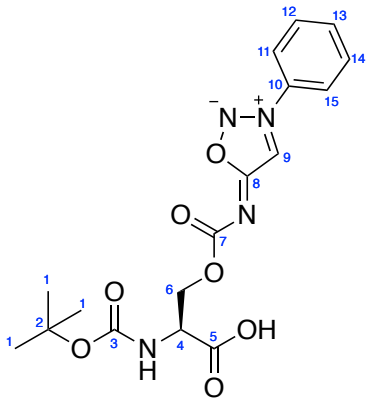
N-(*tert*-butoxycarbonyl)-L-serine *tert*-butyl ester **317** (200 mg, 0.77 mmol) and *N*-((4-nitrophenoxy)carbonyl)-3-phenylsydnonimine **218** (250 mg, 0.77 mmol) were dissolved in acetonitrile (15 mL) and heated under reflux for 24 h. The solvent was removed under reduced pressure and the residue uptaken in dichloromethane (10 mL) and adsorbed onto silica gel. Silica gel chromatography, eluting with CH_2Cl_2 and acetone (100: 0 to 90:10) provided (*S*)-*N*-(((*3*-(*tert-butoxy)-2-*((*tert-butoxycarbonyl)amino)-3-oxopropoxy)carbonyl)-3-phenylsydnon-*imine* **318** (206 mg, 0.46 mmol, 60%) as an orange gum: R_f 0.21 (95:5, CH_2Cl_2:acetone, UV/cerium phosphomolybdate); $[\alpha]_D^{20}$ +34.6, (*c* = 1.0 , $CHCl_3$); ν_{max} (thin film)/cm^{-1} 1722, 1589, 1500, 1458, 1368, 1152, 1030, 972, 846; **^{1}H NMR** (300 MHz; $CDCl_3$) δ 8.17 (1H, s *CH*-11), 7.83 (2H, d, *J* 8.6 Hz, C*H*-13,17), 7.72 (1H, tt, *J* 8.7, 1.2 Hz, C*H*-15) 7.66 (2H, t, *J* 7.9 Hz, C*H*-14,16), 5.51 (1H, d, *J* 8.7, N*H*), 4.55 (1H, dd, *J* 10.9, 3.8 Hz, CH_AH_B-8), 4.46 (1H, m, C*H*-4), 4.31 (1H, dd, *J* 10.9, 3.2 Hz, CH_AH_B-8), 1.45 (9H, s, 3 × CH_3-1) and 1.43 (9H, s, 3 × CH_3-7); **^{13}C NMR** (100 MHz; $CDCl_3$) δ 174.9 (quat., C-9), 169.1 (quat., C-5), 160.5 (quat., C-3), 155.5 (quat., C-9), 133.7 (quat., C-12), 133.3 (*C*H, C-15), 130.6 (*C*H × 2, C-13,17), 121.4 (*C*H × 2, C-14,16), 103.1 (*C*H, C-11), 82.4 (quat., C-2), 79.7 (quat., C-6), 65.9 (CH_2), 53.7 (*C*H), 28.3 (CH_3 × 3, C-1) and 28.1 (CH_3 × 3, C-7); ***m/z*** (ES^+) 471 ($[M+Na]^+$, 100%); **HRMS** *m/z* (ES^+) calcd. for $C_{21}H_{28}N_4NaO_7$ $[M+Na]^+$ requires 471.1856, found 471.1862.

(*S*)-*N*-(((2-Amino-2-carboxyethoxy)carbonyl)-3-phenylsydnonimine trifluoroacetate, 319



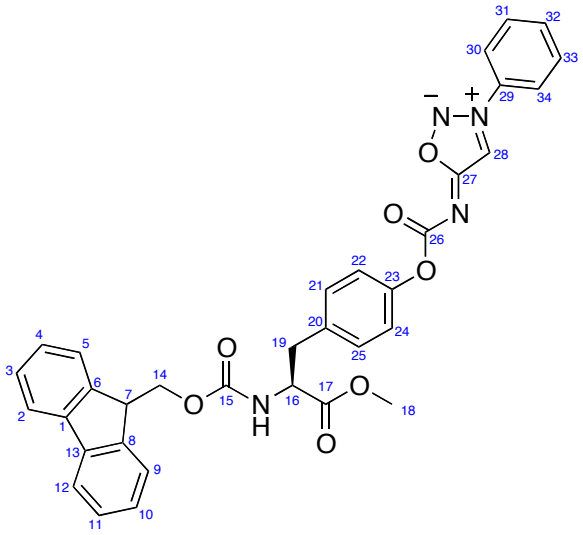
Trifluoroacetic acid (2 mL) was added to a solution of (*S*)-*N*-(((3-(*tert*-butoxy)-2-((*tert*-butoxycarbonyl)amino)-3-oxopropoxy)carbonyl)-3-phenylsydnonimine **318** (200 mg, 0.45 mmol) in dichloromethane (2 mL) at 0 °C. The resultant solution was stirred for 18 h and the solvent evaporated to yield (*S*)-*N*-(((*2-amino-2-carboxyethyl)carbonyl)-3-phenylsydnonimine* **319** (183 mg, 0.45 mmol, quant.) as an orange gum: R_f 0.14 (90:10, CH_2Cl_2:acetone, UV/cerium phosphomolybdate); $[\alpha]_D^{20}$ +3.9, (*c* = 1, MeOH; ν_{max} (NaCl/thin film)/cm^{-1} 1736, 1566, 1487, 1477, 1360, 1010, 956, 822; **^{1}H NMR** (400 MHz, d_6-DMSO) δ 8.62 (1H, s, C*H*-6), 8.51 (3H, s, *br*, N*H*$_2$.CF_3COO*H*), 8.08 (2H, d, *J* 8.0 Hz, C*H*-18,12), 7.81-7.72 (3H, m, C*H*-9,10,11) and 4.42-4.31 (3H, m, CH_2-3, C*H*-1); **^{13}C NMR** (100 MHz, d_6-DMSO) δ 174.5 (quat., C-2), 174.2 (quat., CF_3*C*OOH), 168.8 (quat., C-5), 159.1 (quat., C-4), 133.5 (*C*H, C-10), 130.7 (quat., C-7), 130.2 (*C*H × 2, C-8,12), 122.4 (*C*H × 2, C-9,11), 118.4 (quat., q, *J* 18.0 Hz, *C*F_3COOH), 104.5 (*C*H, C-6), 62.8 (*C*H_2, C-3) and 51.7 (*C*H, C-1); ***m/z*** (ES$^+$) 315 ([M+Na]$^+$, 100%); **HRMS** *m/z* (ES$^+$) calcd. for $C_{12}H_{12}N_4NaO_5$ [M+Na]$^+$ requires 315.0705, found 315.0700.

(*S*)-*N*-((2-((tert-Butoxycarbonyl)amino)-3-oxopropoxy)carbonyl)-3-phenylsydnonimine, 320



Boc_2O (134 mg, 0.62 mmol) and $NaHCO_3$ (52 mg, 0.62 mmol) were added to a solution of (*S*)-*N*-(((2-amino-2-carboxyethoxy)carbonyl)-3-phenylsydnonimine trifluoroacetate **319** (100 mg, 0.246 mmol) in H2O:dioxane (1:1, 2 mL) and stirred overnight at room temperature. The solution was extracted with diethyl ether (2 mL) and the aqueous layer separated. The solution was cooled to 0 °C and acidified (pH = 2) with aq. hydrochloric acid (2 N) with continued stirring. The cooled solution was extracted with ethyl acetate (3 × 2 mL), and the combined organic extracts were washed with brine (2 mL), dried over Na_2SO_4. The mixture was filtered and the solvent removed under reduced pressure to yield (S)-N-((2-((tert-butoxycarbonyl)amino)-3-oxopropoxy)carbonyl)-3-phenylsydnonimine **320** as an orange gum (70 mg, 0.18 mmol, 72%): $\boldsymbol{R_f}$ 0.22 (100: 1, EtOAc:AcOH, UV, $KMnO_4$); $\boldsymbol{[\alpha]_D^{20}}$ +27.2 (*c* = 0.5, MeOH); **^{1}H NMR** (500 MHz, CD_3CN) δ 8.68 (1H, s, C*H*-9), 8.01 (2H, d, J 8.7 Hz, CH-11,13), 7.81-7.72 (3H, m, C*H*-12,13,14), 4.59-4.35 (3H, m, C*H*-4, CH_2-6) and 1.53 (9H, s, 3 × CH_3-1); **^{13}C NMR** (125 MHz, CD_3CN) δ 171.7 (quat., C-8), 170.5 (quat., C-5), 168.6 (quat., C-7), 153.3 (quat., C-3), 134.4 (quat., C-10), 133.0 (*C*H, C-13), 130.7 (*C*H × 2, C-11,15), 122.8 (*C*H × 2, C-12,14), 107.5 (*C*H, C-9), 85.5 (quat., C-2), 61.9 (CH_2, C-6), 55.5 (*C*H, C-4) and 27.5 (CH_3 × 3, C-1); $\boldsymbol{\nu_{max}}$ (NaCl/thin film)/cm^{-1} 1751, 1710, 1630, 1557, 1494, 1451, 1403, 1216, 1050; ***m/z*** (ES^+) 415 ($[M+Na]^+$, 100%); **HRMS** *m/z* (ES^+) calcd. for $C_{17}H_{20}N_4NaO_7$ $[M+Na]^+$ requires 415.1230, found 415.1236.

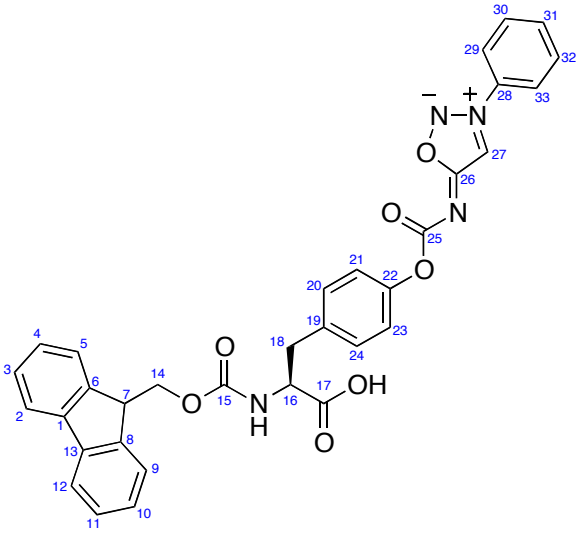
(*S*)-*N*-(((4-(2-(((9*H*-Fluoren-9-yl)methoxy)carbonyl)amino)-3-methoxy-3-oxopropyl)phenoxy)carbonyl)-3-phenylsydnonimine, 321



HCl in dioxane (4M, 2 mL, 8.0 mmol) was added to a solution of (*S*)-*N*-((4-(2-((*tert*-butoxycarbonyl)amino)-3-methoxy-3-oxopropyl)phenyloxy)carbonyl)-3-phenylsydnonimine **298** (320 mg, 0.66 mmol) in dichloromethane (10 mL) cooled to 0 °C. The solution was stirred at 0 °C for 3 h and the solvent evaporated under reduced pressure to yield a white residue which was triturated with diethyl ether. The white powder was suspended in THF (10 mL) and cooled to 0 °C. To this was added Fmoc-Cl (258 mg, 0.99 mmol) and triethylamine (201 mg, 280 µL, 2.00 mmol) and the solution was stirred for 1 hour. The resultant suspension was diluted with ethyl acetate (40 mL). The solution was washed with aq. hydrochloric acid (2 N, 30 mL) and brine (30 mL), dried over Na_2SO_4. The mixture was filtered and the solvent removed under reduced pressure to yield a yellow residue. This was uptaken in dichloromethane (10 mL) and adsorbed onto silica gel. Purification by silica gel chromatography eluting with dichloromethane and acetone (100:0 to 90:10) provided (*S*)-*N*-(((*4-(2-(((9H-fluoren-9-yl)methoxy)carbonyl)amino)-3-methoxy-3-oxopropyl)phenoxy)carbonyl)-3-phenylsydnonimine* **321** (356 mg, 0.59 mmol, 90%) as a white crystalline solid: $\boldsymbol{R_f}$ 0.58 (90:10, CH_2Cl_2:acetone, UV/cerium phosphomolybdate); **m.p.** 74-76 °C; $[\alpha]_D^{20}$ +33.7, (*c* = 1, $CHCl_3$); $\boldsymbol{\nu_{max}}$ (thin film)/cm^{-1} 1742, 1720, 1675, 1587, 1508, 1471, 1450, 1366, 1281, 1213, 1190, 804; **^{1}H NMR** (400 MHz, $CDCl_3$) δ 8.15 (1H, s, C*H*-28), 7.80-7.65 (7H, m, C*H*-2,12,30,31,32,33,34), 7.59 (2H, d, *J* 7.6 Hz, C*H*-5,9), 7.40 (2H, t, *J* 7.5 Hz, C*H*-3,11), 7.31 (2H, tt, *J* 7.5, 1.0 Hz, C*H*-4,10), 7.16 (2H, d, *J* 8.6 Hz, C*H*-21, 25), 7.09 (2H, d, *J* 8.6 Hz, C*H*-22, 24) 5.29 (1H, d, *J* 8.0, N*H*), 4.67 (1H, ddd, *J* 13.8, 8.0, 5.6 Hz, C*H*-16), 4.45 (1H, dd, *J* 10.6, 7.2 Hz, CH_AH_B-14), 4.36 (1H, dd, *J* 10.0, 6.8 Hz, CH_AH_B-14), 4.22 (1H, t, *J* 7.1 Hz, C*H*-7), 3.74 (3H, s, CH_2-18) and 3.12 (2H, *J* 5.7 Hz, CH_2-19); **^{13}C NMR** (100 MHz, $CDCl_3$) δ 175.7 (quat., C-27), 171.9 (quat., C-17), 160.7 (quat., C-26), 155.7 (quat., C-15), 151.2 (quat., C-23), 143.9, 143.8 (quat. × 2, C-6,8),

141.3 (quat. × 2, C-1,13), 133.7 (quat., C-29), 133.3 (*CH*, C-32), 132.5 (quat., C-20), 130.7 (*C*H × 2, C-31,33), 130.1 (*C*H × 2, C-21,25), 127.7 (*C*H × 2, C-3,11), 127.1 (*C*H × 2, C-4,10), 125.2, 125.1 (*C*H × 2, C-2,12), 122.0 (*C*H × 2, C-22,24), 121.6 (*C*H × 2, C-30,34), 120.0 (*C*H × 2, C-2,12), 103.4 (*CH*, C-28), 67.0 (CH_2, C-14), 54.8 (*CH*, C-16), 52.4 (CH_3, C-18), 47.2 (*CH*, C-7) and 37.5 (CH_2, C-19); ***m/z*** (ES^+) 627 ($[M+Na]^+$, 100%); **HRMS** *m/z* (ES^+) calcd. for $C_{34}H_{28}N_4NaO_7$ $[M+Na]^+$ requires 627.1856, found 627.1841.

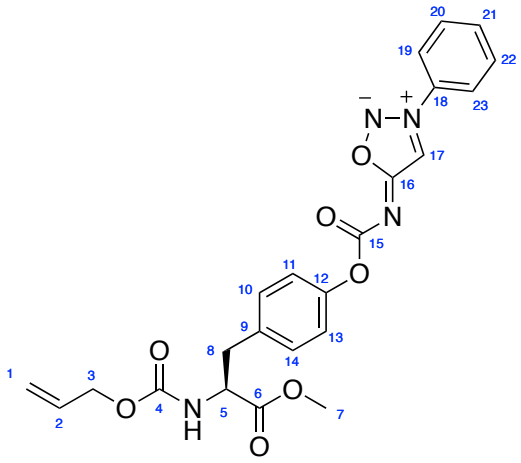
(*S*)-*N*-(((4-(2-(((9*H*-Fluoren-9-yl)methoxy)carbonyl)amino)-2-carboxyethyl)phenoxy)-carbonyl)-3-phenylsydnonimine, 323



Sodium hydroxide (30 mg, 0.75) was added to a solution of (*S*)-*N*-(((4-(2-(((9*H*-fluoren-9-yl)methoxy)carbonyl)amino)-3-methoxy-3-oxopropyl)phenoxy)carbonyl)-3-phenylsydnonimine **321** (218 mg, 0.36 mmol) in THF (5 mL) and water (5 mL). The resulting solution was stirred for 30 min and cooled to 0 °C. The solution was acidified (pH = 2) with aq. hydrochloric acid (2 N) with continued stirring. The cooled solution was extracted with ethyl acetate (3 × 20 mL), and the combined organic extracts were washed with brine (20 mL), dried over Na_2SO_4. The mixture was filtered and the solvent removed under reduced pressure to yield a yellow residue. The residue was uptaken in dichloromethane and adsorbed onto silica gel. Purification by silica gel chromatography, eluting with dichloromethane and methanol (97:3), furnished (*S*)-*N*-(((*4-(2-(((9H-fluoren-9-yl)methoxy)carbonyl)amino)-2-carboxyethyl)phenoxy)carbonyl)-3-phenylsydnonimine* **323** (170 mg, 0.29 mmol, 80%) as a pale yellow solid: R_f 0.29 (90:10, CH_2Cl_2:acetone, UV/cerium phosphomolybdate); **m.p.** 148-150 °C (decomp.), $[\alpha]_D^{20}$ +5.8, (*c* = 0.5, MeOH); ν_{max} (thin film)/cm^{-1} 1712, 1584, 1508, 1471, 1450, 1368, 1296, 1212, 982; **^{1}H NMR** (500 MHz, d_6-DMSO) δ 8.59 (1H, s, C*H*-27), 8.05 (2H, d, *J* 7.8 Hz, C*H*-29,33), 7.87 (2H, d, *J* 6.7 Hz, C*H*-2,12), 7.77 (1H, tt, *J* 7.5, 2.4 Hz, C*H*-31), 7.71 (2H, t, *J* 8.0 Hz, C*H*-30,32), 7.66 (2H, t, *J* 7.9 Hz, C*H*-5,9), 7.41-7.37 (2H, m, C*H*-3,11), 7.33-7.28 (2H, m, C*H*-

4,10), 7.23 (2H, d, *J* 8.2 Hz, C*H*-20,24), 6.99 (2H, d, *J* 8.2 Hz, C*H*-21,23), 4.27-4.23 (1H, m, CH_AH_B-14), 4.20-4.15 (2H, m, C*H*-7, CH_AH_B-14), 4.07 (1H, dd, *J* 13.6, 8.6 Hz, C*H*-16), 3.11 (1H, dd, *J* 13.6, 4.0 Hz CH_AH_B-18) and 2.90 (1H, dd, *J* 13.3, 9.0 Hz, CH_AH_B-18); **^{13}C NMR** (75 MHz, d_6-DMSO) δ 174.9 (quat., C-26), 173.4 (quat., C-17), 158.7 (quat., C-25), 155.6 (quat., C-15), 150.3 (quat., C-22), 143.8 (quat., C-6,8), 140.6 (quat., C-1,13), 135.0 (quat., C-19), 133.6 (quat., C-28), 133.0 (*C*H, C-31), 130.2 (*C*H × 2, C-29,33), 129.9 (*C*H × 2, C-20,24) 127.6 (quat., C-3,11), 127.0 (quat., C-4,10), 125.3, 125.2 (quat., C-5,9), 122.4 (*C*H × 2, C-30,32), 121.2 (*C*H × 2, C-21,23), 120.0 (quat., C-2,12), 104.7 (*C*H, C-27), 65.4 (CH_2, C-14), 56.3 (*C*H, C-16), 46.6 (*C*H, C-7) and 36.3 (CH_2, C-18); ***m/z*** (ES^+) 613 ($[M+Na]^+$, 100%); **HRMS** *m/z* (ES^+) calcd. for $C_{33}H_{26}N_4NaO_7$ $[M+Na]^+$ requires 613.1699, found 613.1677.

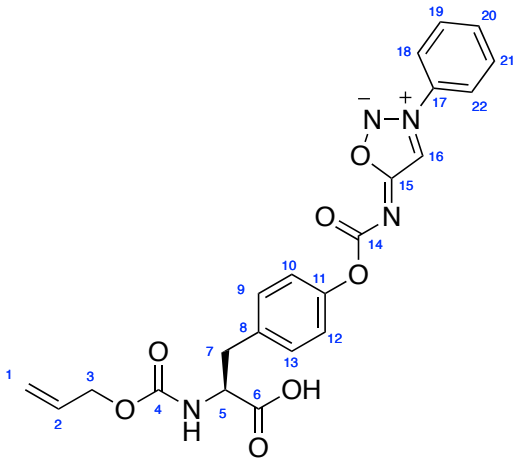
(*S*)-*N*-((4-(2-((Allyloxycarbonyl)amino)-3-methoxy-3-oxopropyl)phenyloxy)-carbonyl)-3-phenylsydnonimine, 322



HCl in dioxane (4M, 0.5 mL, 2.0 mmol) was added to a solution of (*S*)-*N*-((4-(2-((*tert*-butoxycarbonyl)amino)-3-methoxy-3-oxopropyl)phenyloxy)carbonyl)-3-phenylsydnonimine **298** (100 mg, 0.21 mmol) in dichloromethane (5 mL) cooled to 0 °C. The solution was stirred at 0 °C for 3 h and the solvent evaporated under reduced pressure to yield a white residue which was triturated with diethyl ether. The white powder was suspended in THF (8 mL) and cooled to 0 °C. To this was added allyl chloroformate (35 mg, 0.31 mmol) and triethylamine (63 mg, 90 µL, 0.62 mmol) and the solution was stirred for 1 hour. The resultant suspension was diluted with ethyl acetate (40 mL). The solution was washed with aq. hydrochloric acid (2 N, 30 mL) and brine (30 mL), dried over Na_2SO_4. The mixture was filtered and the solvent removed under reduced pressure to yield a yellow residue. This was uptaken in dichloromethane (10 mL) and adsorbed onto silica gel. Purification by silica gel chromatography eluting with dichloromethane and acetone (100:0 to 90:10) provided (*S*)-*N*-((*4*-(*2*-((*allyloxycarbonyl)amino)-3-methoxy-3-oxopropyl)phenyloxy)carbonyl)-3-phenylsydnonimine* **322** (82 mg, 0.18, 85%) as a white solid:

R**f* 0.62 (90:10, CH_2Cl_2:acetone, UV/cerium phosphomolybdate); **m.p.** 101-104 °C; $[\alpha]_D^{20}$ +23.7, (*c* = 1, $CHCl_3$); ν_{max} (NaCl/thin film)/cm^{-1} 1718, 1585, 1508, 1495, 1448, 1403, 1368, 1260, 1213, 1051, 941; **^{1}H NMR** (500 MHz, $CDCl_3$) δ 8.16 (1H, s, C*H*-17), 7.80 (2H, d, *J* 8.4 Hz, C*H*-19,23), 7.76-7.64 (3H, m, C*H*-20,21,22), 7.17-7.10 (4H, m, C*H*-10,11,13,14), 5.96-5.82 (1H, m, C*H*-2), 5.29 (1H, d, *J* 17.1 Hz, C*H*-1-*trans*), 5.21 (1H, d, *J* 10.2 Hz, C*H*-1-*cis*), 4.65 (1H, dd, *J* 13.7, 6.0 Hz, C*H*-5), 4.56 (2H, d, *J* 5.6 Hz, CH_2-3), 3.72 (3H, s, CH_3-7) and 3.11 (2H, d, *J* 5.5 Hz, CH_2-8); **^{13}C NMR** (125 MHz, $CDCl_3$) δ 175.7 (quat., C-16), 172.0 (quat., C-6), 160.4 (quat., C-15), 155.6 (quat., C-4), 151.1 (quat., C-12), 133.7 (*C*H, C-21), 133.4 (quat., C-9), 132.6 (quat., C-18), 132.4 (*C*H, C-2), 130.7 (*C*H × 2, C-19,23), 130.0 (*C*H × 2, C-10,14), 122.0 (*C*H × 2, C-11,13), 121.5 (*C*H × 2, C-20,22), 117.7 (CH_2, C-1), 103.2 (*C*H, C-17), 65.8 (CH_2, C-3), 54.7 (*C*H, C-5), 52.4 (CH_3, C-7) and 37.5 (CH_2, C-8); ***m/z (ES^+) 489 ($[M+Na]^+$, 100%); **HRMS** *m/z* (ES^+) calcd. for $C_{23}H_{22}N_4NaO_7$ $[M+Na]^+$ requires 489.1386, found 489.1392.

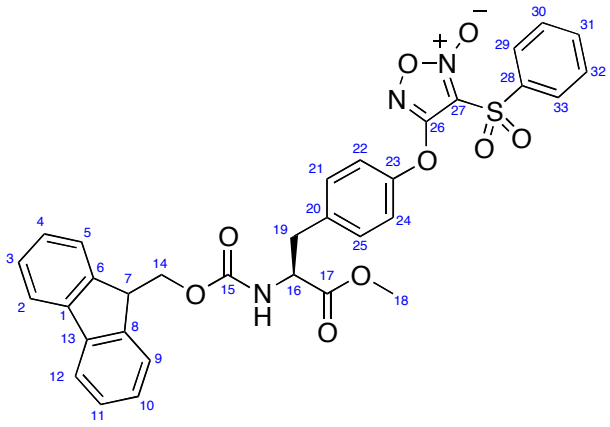
(*S*)-*N*-((4-(2-((Allyloxy)amino)-2-carboxyethyl)phenoxy)carbonyl)-3-phenylsydnonimine, 324



Sodium hydroxide (9 mg, 0.22 mmol) was added to a solution of (*S*)-*N*-((4-(2-((allyloxycarbonyl)amino)-3-methoxy-3-oxopropyl)phenyloxy)carbonyl)-3-phenylsydnonimine **322** (50 mg, 0.11 mmol) in THF (2 mL) and water (2 mL). The resulting solution was stirred for 30 min and cooled to 0 °C. The solution was acidified (pH = 2) with aq. hydrochloric acid (2 N) with continued stirring. The cooled solution was extracted with ethyl acetate (3 × 20 mL), and the combined organic extracts were washed with brine (20 mL), dried over Na_2SO_4. The mixture was filtered and the solvent removed under reduced pressure to yield (*S*)-*N*-((*4*-(*2*-((*allyloxycarbonyl)-2-carboxyethyl)phenoxy)carbonyl) -3-phenylsydnonimine* **324** (39 mg, 0.09 mmol, 80%) as a white solid, which was used without any further purification: ***R**f* 0.28 (90:10, CH_2Cl_2:acetone, UV/cerium phosphomolybdate); **m.p.** 113-116 °C; $[\alpha]_D^{20}$ +26.0, (*c* = 0.5 ,

MeOH; ν_{max} (NaCl/thin film)/cm^{-1} 1647, 1605, 1580, 1494, 1451, 1400, 1256, 1200, 1050; **^{1}H NMR** (500 MHz, CD_3CN) δ 8.57 (1H, s, C*H*-17), 8.06 (2H, d, *J* 7.6 Hz, C*H*-18,22), 7.93 (1H, t, *J* 7.4 Hz, C*H*-20), 7.85 (2H, t, *J* 8.2 Hz, C*H*-19,21), 7.41 (2H, d, *J* 8.4 Hz, C*H*-9,13), 7.24 (2H, d, *J* 8.4 Hz, C*H*-9,13), 6.06-5.92 (2H, m, C*H*-2, N*H*), 5.38 (1H, d, *J* 17.7 Hz, C*H*-1-*trans*), 5.29 (1H, d, *J* 10.3 Hz, C*H*-1-*cis*), 4.63-4.57 (1H, m, CH_2-3), 4.54 (1H, ddd, *J* 14.0, 9.0, 5.0 Hz, C*H*-5), 3.33 (1H, dd, *J* 14.0, 5.4 Hz, CH_AH$_B$-7) and 3.08 (1H, dd, *J* 14.0, 9.0 Hz, CH$_A$*H*$_B$-7); **^{13}C NMR** (125 MHz, CD_3CN) δ 173.0 (quat., C-15), 172.0 (quat., C-6), 163.2 (quat., C-14), 155.9 (quat., C-4), 151.6 (quat., C-11), 136.2 (quat., C-8), 133.3 (*C*H, C-2), 130.2 (*C*H × 2, C-9,13), 133.6 (quat., C-17), 133.5 (*C*H, C-20), 130.5 (*C*H × 2, C-18,22), 122.5 (*C*H × 2, C-19,21), 121.7 (*C*H × 2, C-10,12), 116.6 (*C*H$_2$, C-1), 102.7 (*C*H, C-16), 65.2 (*C*H$_3$, C-3), 55.2 (*C*H, C-5) and 36.3 (*C*H$_2$, C-7); ***m/z*** (ES$^+$) 475 ([M+Na]$^+$, 100%); **HRMS** *m/z* (ES$^+$) calcd. for $C_{22}H_{20}N_4NaO_7$ [M+Na]$^+$ requires 475.1230, found 475.1238.

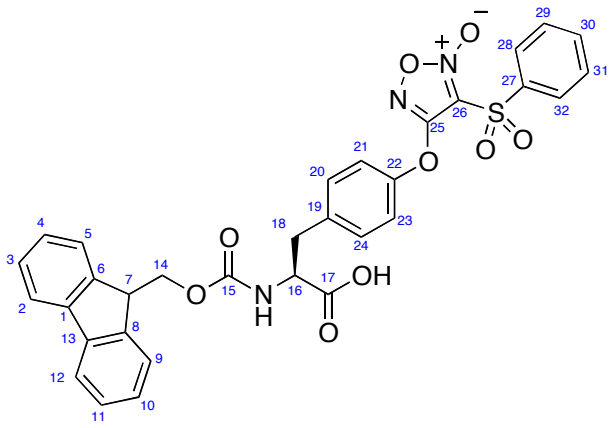
(*S*)-4-(4-(2-((((9H-Fluoren-9-yl)methoxy)carbonyl)amino)-3-methoxy-3-oxopropyl)phenoxy)-3-(phenylsulfonyl)-1,2,5-oxadiazole 2-oxide, 325



HCl in dioxane (4M, 2 mL, 8.0 mmol) was added to a solution of (*S*)-4-(4-(2-((*tert*-butoxycarbonyl)amino)-3-methoxy-3-oxopropyl)phenoxy)-3-(phenylsulfonyl)-1,2,5-oxadiazole 2-oxide **300** (500 mg, 0.96 mmol) in dichloromethane (10 mL) cooled to 0 °C. The solution was stirred at 0 °C for 3 h and the solvent evaporated under reduced pressure to yield a white residue which was triturated with diethyl ether. The white powder was suspended in THF (30 mL) and cooled to 0 °C. To this was added Fmoc-Cl (374 mg, 1.45 mmol) and triethylamine (292 mg, 410 μL, 2.00 mmol) the solution was stirred for 1 hour. The resultant suspension was diluted with ethyl acetate (20 mL). The solution was washed with aq. hydrochloric acid (2 N, 30 mL) and brine (30 mL), dried over Na_2SO_4. The mixture was filtered and the solvent removed under reduced pressure to yield a white residue. This was uptaken in dichloromethane and adsorbed onto silica gel. Purification by silica gel chromatography eluting with ethyl acetate and hexanes

(0:100 to 15:85) provided (*S*)-*4*-(*4*-(*2*-((((*9H-fluoren-9-yl)methoxy)carbonyl)amino)-3-methoxy-3-oxopropyl)phenoxy)-*3*-(*phenylsulfonyl*)-1,2,*5*-*oxadiazole 2-oxide* **325** (556 mg, 0.92 mmol, 90%) as a white solid: R_f 0.50 (50:50, EtOAc:PE, UV/cerium phosphomolybdate); **m.p.** 141-143 °C; $[\alpha]_D^{20}$ +25.4, (*c* = 1, $CHCl_3$); ν_{max} (thin film)/cm^{-1} 1746, 1692, 1625, 1539, 1506, 1450, 1357, 1260, 1165; **^{1}H NMR** (500 MHz, $CDCl_3$) δ 8.10 (2H, d, *J* 8.3 Hz, C*H*-28, 32), 7.79-7.76 (3H, m, C*H*-2,12, 30), 7.63 (2H, t, *J* 8.2 Hz, C*H*-29,31), 7.57 (2H, dd, *J* 7.4, 4.4 Hz, C*H*-5,9), 7.40 (2H, t, *J* 7.2 Hz, C*H*-3,11), 7.32 (2H, t, *J* 7.6 Hz, C*H*-4,10). 7.22 (2H, d, *J* 8.5 Hz, C*H*-21, 25), 7.15 (2H, d, *J* 8.5 Hz, C*H*-22, 24), 5.34 (1H, d, *J* 8.1, N*H*), 4.67 (1H, dd, *J* 13.6, 6.0 Hz, C*H*-16), 4.47 (1H, dd, *J* 10.6, 7.2 Hz, CH_AH_B-14), 4.37 (1H, dd, *J* 10.2, 6.7 Hz, CH_AH_B-14), 4.20 (1H, t, *J* 5.8 Hz, C*H*-7), 3.73 (3H, s, CH_2-18), 3.18 (1H, dd, *J* 14.0, 5.8 Hz, CH_AH_B-19) and 3.09 (1H, dd, *J* 14.0, 6.2 Hz, CH_AH_B-19); **^{13}C NMR** (125 MHz, $CDCl_3$) δ 171.7 (quat., C-17) 158.3 (quat., C-26), 155.6 (quat., C-15), 151.6 (quat., C-23), 144.0, 143.7 (quat., × 2, C-6,8), 141.3 (quat., C-1, 14), 137.9 (quat., C-28), 135.9 (*C*H, C-31), 130.9 (*C*H × 2, C-21,25), 129.8 (*C*H × 2, C-29,33), 128.7 (*C*H × 2, C-30,32), 127.8 (*C*H × 2, C-3,11), 127.1 (*C*H × 2, C-4,10), 125.1, 125.0 (*C*H × 2, C-5,9), 120.1 (*C*H × 2, C-2,12), 119.9 (*C*H × 2, C-22, 24) 110.7 (quat., C-27) and 66.9 (CH_2, C-14), 54.7 (*C*H, C-16), 52.5 (CH_3, C-18), 47.2 (*C*H, C-7) and 37.7 (CH_2, C-19); ***m/z*** (ES^+) 664 ($[M+Na]^+$, 100%); **HRMS** *m/z* (ES^+) calcd. for $C_{33}H_{27}N_3NaO_9S$ $[M+Na]^+$ requires 664.1366, found 664.1359.

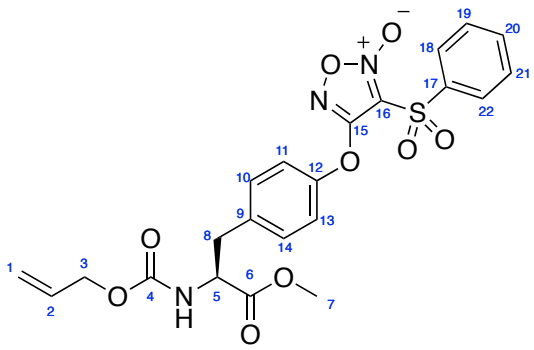
(*S*)-4-(4-(2-((((9H-Fluoren-9-yl)methoxy)carbonyl)amino)-3-oxopropyl)phenoxy)-3-(phenylsulfonyl)-1,2,5-oxadiazole 2-oxide, 327



Sodium hydroxide (30 mg, 0.75) was added to a solution of ((*S*)-4-(4-(2-((((9H-fluoren-9-yl)methoxy)carbonyl)amino)-3-methoxy-3-oxopropyl)phenoxy)-3-(phenylsulfonyl)-1,2,5-oxadiazole 2-oxide **325** (226 mg, 0.35 mmol) in THF (5 mL) and water (5 mL). The resulting solution was stirred for 30 min and cooled to 0 °C. The solution was acidified (pH = 2) with aq. hydrochloric acid (2 N) with continued stirring. The cooled solution was extracted with ethyl

acetate (3 × 20 mL), and the combined organic extracts were washed with brine (20 mL), dried over Na_2SO_4. The mixture was filtered and the solvent removed under reduced pressure to yield a yellow residue. The residue was uptaken in dichloromethane and adsorbed onto silica gel. Purification by silica gel chromatography, eluting with dichloromethane and methanol (97:3), furnished (*S*)-*4*-(*4*-(*2*-((((*9H-fluoren-9-yl)methoxy)carbonyl)amino)-3-oxopropyl)phenoxy)-3-(*phenylsulfonyl)*-1,2,*5-oxadiazole 2-oxide* **325** (177 mg, 0.28 mmol, 80%) as a white solid; $\boldsymbol{R_f}$ 0.33 (100:1, EtOAc:AcOH, UV/cerium phosphomolybdate); **m.p.** 189-191 °C; $[\boldsymbol{\alpha}]_{\mathbf{D}}^{\mathbf{20}}$ +3.9, (*c* = 0.5, MeOH; $\boldsymbol{\nu_{max}}$ (thin film)/cm^{-1} 1689, 1623, 1537, 1501, 1446, 1353, 1161, 1022; **^{1}H NMR** (500 MHz, d_6-DMSO) δ 8.04 (2H, d, *J* 7.7 Hz, C*H*-28, 32), 7.90 (1H, t, *J* 7.4 Hz, C*H*-30), 7.86 (2H, d, *J* 7.5 Hz, C*H*-2,12), 7.79-7.74 (3H, m, C*H*-29, 31, N*H*-Fmoc), 7.63 (2H, t, *J* 7.7 Hz, C*H*-5,9), 7.41-7.34 (4H, m, C*H*-3,11,20,24), 7.32-7.27 (4H, m, C*H*-4,10, 21, 23), 4.22-4.15 (4H, m, C*H*-7, 16, CH_2-14), 3.13 (1H, dd, *J* 13.9, 4.4 Hz, CH_AH$_B$-18) and 3.13 (1H, dd, *J* 13.6, 10.9 Hz, CH$_A$*H*$_B$-18); **^{13}C NMR** (125 MHz, d_6-DMSO) δ 173.7 (quat., C-17) 159.0 (quat., C-25), 156.5 (quat., C-15), 151.7 (quat., C-22), 144.2 (quat., × 2, C-6,8), 141.2 (quat., C-1,13), 137.9 (quat., C-27), 136.8 (*C*H, C-30), 131.1 (*C*H × 2, C-20,24), 130.5 (*C*H × 2, C-28,32), 129.0 (*C*H × 2, C-29,31), 128.1 (*C*H × 2, C-3,11), 127.6 (*C*H × 2, C-4,10), 125.7 (*C*H × 2, C-5,9), 120.6 (*C*H × 2, C-2,12), 119.9 (*C*H × 2, C-21,23) 111.7 (quat., C-26), 66.1 (*C*H$_2$, C-14), 55.8 (*C*H, C-16), 47.0 (*C*H, C-7) and 36.2 (*C*H$_2$, C-19); ***m/z*** (ES$^+$) 650 ([M+Na]$^+$, 100%); **HRMS** *m/z* (ES$^+$) calcd. for $C_{32}H_{25}N_3NaO_9S$ [M+Na]$^+$ requires 650.1209, found 650.1215.

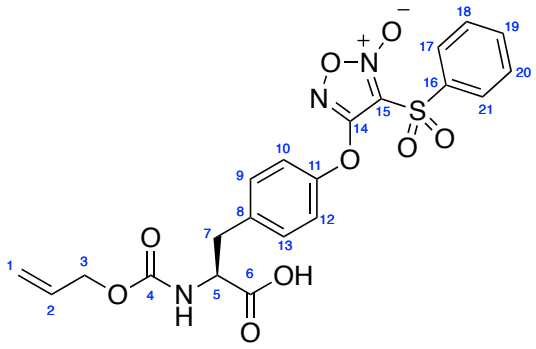
(*S*)-4-(4-(2-((Allyloxycarbonyl)amino)-3-methoxy-3-oxopropyl)phenoxy)-3-(phenylsulfonyl)-1,2,5-oxadiazole 2-oxide, 326



HCl in dioxane (4M, 0.5 mL, 2.0 mmol) was added to a solution of (*S*)-4-(4-(2-((*tert*-butoxycarbonyl)amino)-3-methoxy-3-oxopropyl)phenoxy)-3-(phenylsulfonyl)-1,2,5-oxadiazole 2-oxide **300** (227 mg, 0.44 mmol) in dichloromethane (5 mL) cooled to 0 °C. The solution was stirred at 0 °C for 3 h and the solvent evaporated under reduced pressure to yield a white residue which was triturated with diethyl ether. The white powder was suspended in THF (12 mL) and

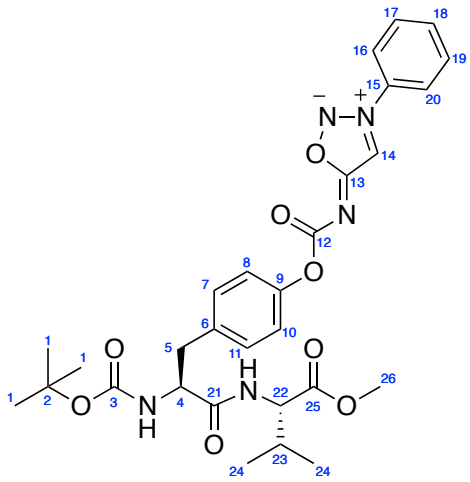
cooled to 0 °C. To this was added allyl chloroformate (80 mg, 70 μL, 0.66 mmol) and triethylamine (132 mg, 180 μL, 1.31 mmol) the solution was stirred for 1 hour. The resultant suspension was diluted with ethyl acetate (20 mL). The solution was washed with aq. hydrochloric acid (2 N, 30 mL) and brine (30 mL), dried over Na_2SO_4. The mixture was filtered and the solvent removed under reduced pressure to yield a white residue. This was uptaken in dichloromethane and adsorbed onto silica gel. Purification by silica gel chromatography eluting with ethyl acetate and hexanes (0:100 to 15:85) provided (*S*)-*4*-(*4*-(*2*-((*allyloxycarbonyl)amino)-3-methoxy-3-oxopropyl)phenoxy)-3-(*phenylsulfonyl*)-1,2,*5-oxadiazole 2-oxide* **326** (178 mg, 0.35 mmol, 85%) as a white solid: $\boldsymbol{R_f}$ 0.62 (50:50, EtOAc:PE, UV/cerium phosphomolybdate); **m.p.** 78-80 °C; $[\boldsymbol{\alpha}]_{\mathbf{D}}^{\mathbf{20}}$ +29.3, (*c* = 1, $CHCl_3$); $\boldsymbol{\nu_{max}}$ (NaCl/thin film)/cm^{-1} 1745, 1614, 1536, 1505, 1449, 1439, 1314, 1257, 1211, 1167, 1019; **^{1}H NMR** (500 MHz, $CDCl_3$) δ 8.09 (2H, d, *J* 8.3 Hz, C*H*-18,22), 7.79 (1H, tt, *J* 6.7, 1.2 Hz, C*H*-20), 7.64 (2H, t, *J* 7.9 Hz, C*H*-19,21), 7.31-7.22 (4H, m, C*H*-10, 11, 13, 14), 5.94-5.82 (1H, m, C*H*-2), 5.28 (1H, dd, *J* 17.2, 1.2 Hz, C*H*-1-*trans*), 5.21 (1H, dd, *J* 10.2, 1.2 Hz, C*H*-1-*cis*), 4.68-4.35 (3H, m, CH_2-3, C*H*-5), 3.72 (3H, s, CH_3-7) and 3.21-3.05 (2H, m, CH_2-8); **^{13}C NMR** (125 MHz, $CDCl_3$) δ 171.7 (quat., C-6), 158.3 (quat., C-15), 155.5 (quat., C-4), 151.6 (quat., C-12), 137.9 (quat., C-17), 135.9 (*C*H, C-20), 134.7 (quat., C-9), 132.5 (*C*H, C-2), 130.8 (*C*H × 2, C-10,14), 129.8 (*C*H × 2, C-18,22), 128.6 (*C*H × 2, C-19,21), 119.9 (*C*H × 2, C-11,13), 118.0 (*C*H_2, C-1), 110.7 (quat., C-16), 65.9 (*C*H_2, C-3), 54.7 (*C*H, C-5), 52.5 (*C*H_3, C-7) and 37.7 (*C*H_2, C-8); ***m/z*** (ES^+) 526 ($[M+Na]^+$, 100%); **HRMS** *m/z* (ES^+) calcd. for $C_{22}H_{21}N_3NaO_9S$ $[M+Na]^+$ requires 526.0896, found 526.0902.

(*S*)-4-(4-(2-((Allyoxycarbonyl)amino)-2-carboxyethyl)phenoxy)-3-(phenylsulfonyl)-1,2,5-oxadiazole 2-oxide, 328



Sodium hydroxide (16 mg, 0.40 mmol) was added to a solution of (*S*)-4-(4-(2-((allyloxycarbonyl)amino)-3-methoxy-3-oxopropyl)phenoxy)-3-(phenylsulfonyl)-1,2,5-oxadiazole 2-oxide **326** (100 mg, 0.20 mmol) in THF (2 mL) and water (2 mL). The resulting solution was stirred for 30 min and cooled to 0 °C. The solution was acidified (pH = 2) with aq. hydrochloric acid (2 N) with continued stirring. The cooled solution was extracted with ethyl acetate (3 × 20 mL), and the combined organic extracts were washed with brine (20 mL), dried over Na_2SO_4. The mixture was filtered and the solvent removed under reduced pressure to yield (*S*)-*4*-(*4*-(*2*-((*Allyoxycarbonyl)amino)-2-carboxyethyl)phenoxy)-3-(phenylsulfonyl)*-1,2,5-oxadiazole 2-oxide* **328** (87 mg, 0.18 mmol, 90%) as a white solid, which was used without any further purification: $\boldsymbol{R_f}$ 0.41 (100:1, EtOAc:AcOH, UV/cerium phosphomolybdate); **m.p.** 138-141 °C; $[\boldsymbol{\alpha}]_{\mathbf{D}}^{\mathbf{20}}$ +23.5, (*c* = 0.7, MeOH; $\boldsymbol{\nu_{max}}$ (NaCl/thin film)/cm^{-1} 2525, 1658, 1537, 1451, 1404, 1260, 1201, 1112, 1020; **^{1}H NMR** (500 MHz, CD_3CN) δ 8.18 (2H, d, *J* 7.9 Hz, C*H*-17,21), 7.98 (1H, t, *J* 7.6 Hz, C*H*-19), 7.83 (2H, t, *J* 7.9 Hz, C*H*-18,20), 7.47-7.43 (2H, m, C*H*-9,13) 7.36 (2H, d, *J* 7.5 Hz, C*H*-10,12), 6.05-5.95 (2H, m, C*H*-2, N*H*), 5.36 (1H, d, *J* 17.7 Hz, C*H*-1-*trans*), 5.28 (1H, dd, *J* 10.7 Hz, C*H*-1-*cis*), 4.59 (2H, d, *J* 5.3 Hz, CH_2-3), 4.54 (1H, ddd, *J* 15.0, 9.1, 5.0 Hz, C*H*-5), 3.34 (1H, ddd, *J* 15.0, 5.0 Hz, CH_AH_B-7) and 3.09 (1H, ddd, *J* 15.0, 9.1 Hz, CH_AH_B-7); **^{13}C NMR** (125 MHz, CD_3CN) δ 172.2 (quat., C-6), 158.9 (quat., C-14), 155.5 (quat., C-4), 151.7 (quat., C-11), 137.6 (quat., C-16), 136.1 (*C*H, C-19), 136.0 (quat., C-8), 133.2 (*C*H, C-2), 131.0 (*C*H × 2, C-9,13), 129.9 (*C*H × 2, C-17,21), 128.7 (*C*H × 2, C-18,20), 119.9 (*C*H × 2, C-10,12), 116.6 (CH_2, C-1), 111.3 (quat., C-15), 65.1 (CH_2, C-3), 54.9 (*C*H, C-5) and 36.2 (CH_2, C-7); ***m/z*** (ES^+) 512 ($[M+Na]^+$, 100%); **HRMS** *m/z* (ES^+) calcd. for $C_{21}H_{19}N_3NaO_9S$ $[M+Na]^+$ requires 512.0740 found 512.0745.

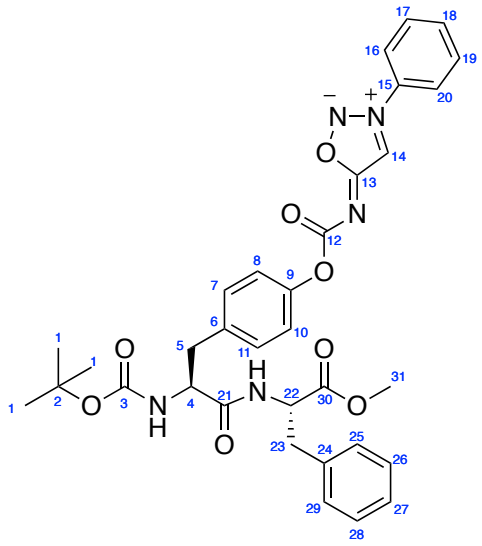
Boc-Tyr(*O*-carbonyl-*N*-3-phenylsydnonimine)-Val-OMe, 329



HATU (442 mg, 1.16 mmol) was added to a solution of valine methyl ester hydrochloride (49 mg, 0.35 mmol) and (*S*)-*N*-((4-(2-((*tert*-butoxycarbonyl)amino)-2-carboxyethyl)phenoxy)carbonyl)-3-phenylsydnonimine **308** (150 mg, 0.32 mmol) in dry DMF (5 mL) cooled to 0 °C. The solution was stirred for 15 min and diisopropylethylamine (distilled, 200 mg, 270 µL, 1.55 mmol) was added. The solution was allowed to warm to room temperature and stirred for 18 h. The resultant solution was diluted with ethyl acetate (50 mL) and water (50 mL). The organic layer was separated and washed with aq. hydrochloric acid (2 N, 20 mL), saturated $NaHCO_3$ (20 mL) and brine (20 mL). The organic layer was separated and dried over Na_2SO_4, filtered and the solvent removed under reduced pressure. The resultant residue was uptaken in dichloromethane and adsorbed onto silica gel. Purification by silica gel chromatography, eluting with dichloromethane and methanol (100:0 to 97:3) provided *Boc-Tyr*(*O-carbonyl-N-3-phenylsydnonimine)-Val-OMe* **329** (169 mg g, 0.29 mmol, 91%) as a white solid: $\boldsymbol{R_f}$ 0.62 (90:10, CH_2Cl_2:MeOH, UV/cerium phosphomolybdate); **m.p.** 139-140 °C; $[\boldsymbol{\alpha}]_{\mathbf{D}}^{\mathbf{20}}$ +29.4, (*c* = 1, $CHCl_3$); $\boldsymbol{\nu_{max}}$ (NaCl/thin film)/cm^{-1} 1743, 1647, 1593, 1545, 1495, 1451, 1367, 1251, 1215, 1050: **^{1}H NMR** (500 M, $CHCl_3$) δ 8.17 (1H, s, C*H*-14), 7.79 (2H, d, *J* 7.9 Hz, C*H*-16,20), 7.69 (1H, tt, *J* 7.5, 1.2 Hz, C*H*-18), 7.69 (2H, t, *J* 7.7 Hz, C*H*-17,19), 7.68 (2H, d, *J* 7.5 Hz, C*H*-7,11), 7.16 (2H, d, *J* 7.8 Hz, C*H*-8,10), 7.09 (2H, d, *J* 8.40, C*H*-8,10), 6.55 (1H, s, *br*, N*H*-amide), 5.01 (1H, s, *br*, N*H*-Boc), 4.43 (1H, dd, *J* 8.6, 5.1 Hz, C*H*-22), 4.36-4.28 (1H, s, C*H*-4), 3.66 (3H, s, CH_3-26), 3.07-2.93 (2H, m, CH_2-5), 2.10-2.04 (1H, m, C*H*-23), 1.38 (9H, s, 3 × CH_3-1), 0.85 (3H, d, *J* 7.0 Hz, CH_3-24) and 0.82 (3H, d, *J* 7.0 Hz, CH_3-24); **^{13}C NMR** (125 M, $CHCl_3$) δ 175.7 (quat., C-13), 171.8 (quat., C-25), 171.2 (quat., C-21), 160.3 (quat., C-12), 155.5 (quat., C-3), 151.0 (quat., C-9), 133.7 (quat., C-6), 133.4 (quat., C-15), 133.3 (*C*H, C-18), 130.6 (*C*H × 2, C-16, 20), 130.1 (*C*H × 2, C-7,11), 121.9 (*C*H × 2, C-8,10), 121.3 (*C*H × 2, C-17,19), 103.4 (*C*H, C-14), 80.2 (quat., C-2), 57.3 (*C*H, C-22), 55.8 (*C*H, C-4), 52.1 (*C*H_3, C-26),

37.1 (CH_2, C-5), 31.2 (*C*H, C-23), 28.3 (CH_3 × 3, C-1) and 18.8, 17.8 (CH_3 × 2, C-24); ***m/z*** (ES^+) 604 ($[M+Na]^+$, 100%); **HRMS** *m/z* (ES^+) calcd. for $C_{29}H_{35}N_5NaO_8$ $[M+Na]^+$ requires 604.2383, found 604.2395.

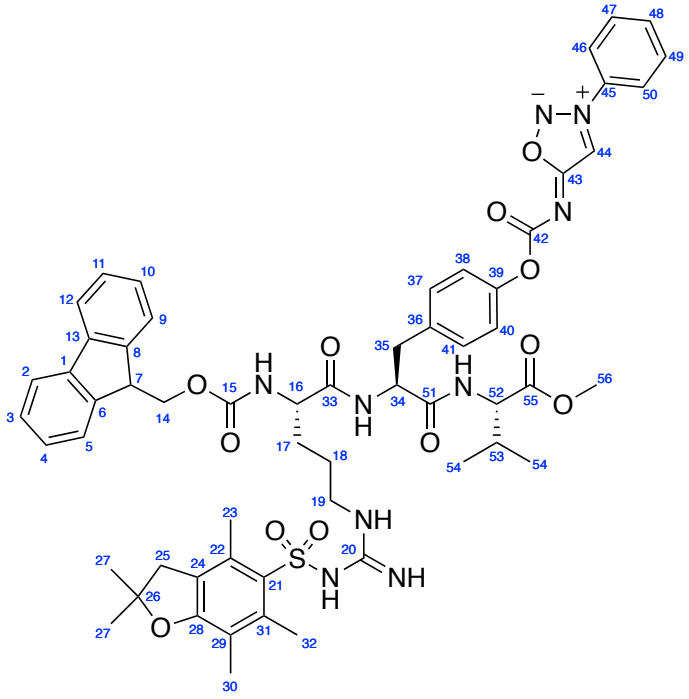
Boc-Tyr(*O*-carbonyl-*N*-3-phenylsydnonimine)-Phe-OMe, 330



HATU (442 mg, 1.16 mmol) was added to a solution of phenylalanine methyl ester hydrochloride (76 mg, 0.352 mmol) and (*S*)-*N*-((4-(2-((*tert*-butoxycarbonyl)amino)-2-carboxyethyl)phenoxy)carbonyl) -*3-phenylsydnonimine* **308** (150 mg, 0.32 mmol) in dry DMF (5 mL) cooled to 0 °C. The solution was stirred for 15 min and diisopropylethylamine (distilled, 200 mg, 270 µL, 1.55 mmol) was added. The solution was allowed to warm to room temperature and stirred for 18 h. The resultant solution was diluted with ethyl acetate (50 mL) and water (50 mL). The organic layer was separated and washed with aq. hydrochloric acid (2 N, 20 mL), saturated $NaHCO_3$ (20 mL) and brine (20 mL). The organic layer was separated and dried over Na_2SO_4, filtered and the solvent removed under reduced pressure. The resultant residue was uptaken in dichloromethane and adsorbed onto silica gel. Purification by silica gel chromatography, eluting with ethyl acetate and hexanes (10:90 to 25:75) provided *Boc-Tyr*(*O-carbonyl-N-3-phenylsydnonimine)-Phe-OMe* **330** (191 mg, 0.30 mmol, 95%) as a fawn solid: $\boldsymbol{R_f}$ 0.58 (90:10, CH_2Cl_2:MeOH, UV/cerium phosphomolybdate); **m.p.** 159-160 °C; $[\alpha]_D^{20}$ +17.6, (*c* = 0.7 , $CHCl_3$); $\boldsymbol{\nu_{max}}$ (NaCl/thin film)/cm^{-1} 1733, 1677, 1587, 1508, 1438, 1368, 1215, 1167, 1050; **^{1}H NMR** (500 MHz, $CHCl_3$) δ 8.27 (1H, s, C*H*-14), 7.85 (2H, d, *J* 7.7 Hz, C*H*-16,20), 7.76 (1H, t, *J* 7.4 Hz, C*H*-18), 7.76 (2H, t, *J* 7.7 Hz, C*H*-17, 19), 7.30-7.14 (7H, m, C*H*-7,11,25,26,27,28,29), 7.07 (2H, d, *J* 7.6 Hz, C*H*-8,10), 6.55 (1H, d, *J* 7.9, N*H*-amide), 5.01 (1H, d, *J* 8.1, N*H*-Boc), 4.82 (1H, dd, *J* 13.4, 6.3 Hz, C*H*-22), 4.40-4.33 (1H, m, C*H*-4), 3.70 (3H, s, CH_3-31), 3.14-3.03 (4H, m, CH_2-5, 23), and 1.43 (9H, s, 3 × *CH*$_3$-1); **^{13}C NMR** (125 MHz,

$CHCl_3$) δ 175.7 (quat., C-13), 171.4 (quat., C-30), 170.9 (quat., C-21), 160.2 (quat., C-12), 155.3 (quat., C-3), 151.0 (quat., C-9), 135.8 (*C*H, C-27), 129.2 (*C*H × 2, C-25, 29), 128.5 (*C*H × 2, C-26, 28), 127.1 (quat., C-24), 121.9 (*C*H × 2, C-8,10), 121.6 (*C*H × 2, C-17, 19), 133.7 (quat., C-6), 133.3 (*C*H, C-18), 133.3 (quat., C-15), 130.6 (*C*H × 2, C-16, 20), 130.1 (*C*H × 2, C-7, 11), 103.5 (*C*H, C-14), 80.2 (quat., C-2), 55.6 (*C*H, C-4), 53.4 (*C*H, C-22), 52.3 (CH_3, C-31), 37.9 (CH_2, C-23), 37.4 (CH_2, C-5) and 28.2 (CH_3 × 3, C-1); ***m/z*** (ES^+) 652 ($[M+Na]^+$, 100%); **HRMS** *m/z* (ES^+) calcd. for $C_{33}H_{35}N_5NaO_8$ $[M+Na]^+$ requires 652.2383, found 652.2390.

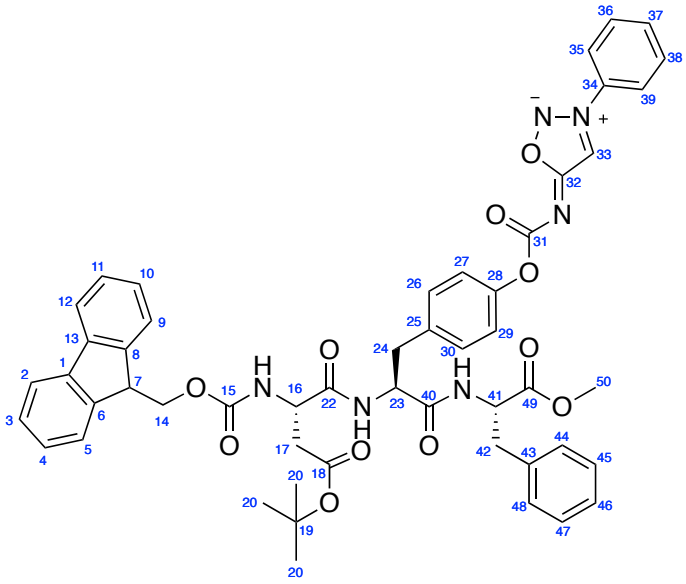
Fmoc-Arg(Pbf)-Tyr(*O*-carbonyl-*N*-3-phenylsydnonimine)-Val-OMe, 331



HCl in dioxane (4M, 3 mL, 12.0 mmol) was added to a solution of dipeptide **329** (186 mg, 0.32 mmol) in dichloromethane (10 mL) cooled to 0 °C. The solution was stirred at 0 °C for 3 h and the solvent evaporated under reduced pressure to yield a white residue which was triturated with diethyl ether. The residue was dissolved in dry DMF (5 mL) and Fmoc-Arg(Pbf)-OH (208 mg, 0.32 mmol) was added. The solution was cooled to 0 °C and stirred for 10 min. HATU (442 mg, 1.297 mmol) was added and the solution stirred for a further 15 min. Diisopropylethylamine (distilled, 166 mg, 220 µL, 1.28 mmol) was added and the solution was allowed to warm to room temperature and stirred for 18 h. The resultant solution was diluted with ethyl acetate (50 mL) and water (50 mL). The organic layer was separated and washed with aq. hydrochloric acid (2 N, 20 mL), saturated $NaHCO_3$ (20 mL) and brine (20 mL). The organic layer was separated and dried over Na_2SO_4, filtered and the solvent removed under reduced pressure. The resultant

residue was uptaken in dichloromethane and adsorbed onto silica gel. Purification by silica gel chromatography, eluting with dichloromethane and methanol (99:1) provided *Fmoc-Arg(Pbf)-Tyr*(*O-carbonyl-N-3-phenylsydnonimine)-Val-OMe* **331** (312 mg, 0.28 mmol, 85%) as a white solid: $\boldsymbol{R_f}$ 0.59 (90:10, CH_2Cl_2:MeOH, UV/cerium phosphomolybdate); **m.p.** 115-119 °C; $[\boldsymbol{\alpha}]_{\boldsymbol{D}}^{\boldsymbol{20}}$+110 (*c* = 0.6 , $CHCl_3$); $\boldsymbol{\nu_{max}}$ (NaCl/thin film)/cm^{-1} 1725, 1651, 1553, 1451, 1404, 1369, 1212, 1107, 1019, 974, 851; **^{1}H NMR** (500 MHz, $CDCl_3$) δ 8.27 (1H, s, C*H*-44), 7.79 (2H, d, *J* 7.7 Hz, C*H*-46,50), 7.75-7.67 (2H, m, C*H*-2,12), 7.63 (1H, t, *J* 7.5 Hz, C*H*-48), 7.55 (2H, t, *J* 7.9 Hz, C*H*-47,49), 7.52 (2H, d, *J* 7.5 Hz, C*H*-5,9), 7.30 (2H, t, *J* 7.5 Hz, C*H*-3,11), 7.21-7.16 (4H, m, C*H*-4,10,37,41), 7.10 (1H, s, *br*, N*H*-sulfonamide), 7.02 (2H, d, *J* 8.0 Hz, C*H*-38,40), 6.54-6.15 (5H, m, 2 × N*H*-amide, 2 × N*H*-guanidine, N*H*-Fmoc), 4.74 (1H, dd, *J* 14.4, 8.0 Hz, C*H*-34), 4.36 (1H, dd, *J* 7.5, 5.8 Hz, C*H*-52), 4.31-4.23 (3H, m, C*H*-16, CH_2-14), 4.07 (1H, t, *J* 6.8 Hz, C*H*-7), 3.62 (3H, s, CH_3-56), 3.26-3.10 (3H, m, CH_2-17, CH_AH_B-35), 3.04-2.98 (1H, m, CH_AH_B-35), 2.86 (2H, s, CH_2-25), 2.58 (CH_3, s, CH_3-23), 2.50 (3H, s, CH_3-30), 2.05-2.01 (4H, m, CH_3-32, C*H*-53), 1.73-1.65 (1H, m, CH_AH_B-19), 1.60-1.53 (1H, m, CH_AH_B-19), 1.41-1.35 (8H, m, CH_2-18, 3 × CH_3-27) and 0.83-0.80 (6H, m, 2 × CH_3-54); **^{13}C NMR** (125 MHz, $CDCl_3$) δ 175.5 (quat., C-43), 172.9 (quat., C-33), 171.8 (quat., C-55), 171.4 (quat., C-51), 159.9 (quat., C-42), 158.7 (quat., C-20), 156.6 (quat., C-15), 156.4 (quat., C-28), 150.6 (quat., C-39), 144.0, 143.6 (quat. × 2, C-1,13), 141.2, 141.1 (quat. × 2, C-6,8), 138.4 (quat., C-21), 133.9 (quat., C-36), 133.6 (quat., C-45), 133.2 (*C*H, C-48), 133.0 (quat., C-24), 132.3 (quat., C-29), 130.5 (*C*H × 2, C-46,50), 130.2 (*C*H × 2, C-37,41), 127.7 (*C*H × 2, C-1,13), 127.1 (*C*H × 2, C-4,10), 125.3, 125.2 (*C*H × 2, C-5,9), 124.6 (quat., C-31), 121.8 (*C*H × 2, C-38,40), 121.6 (*C*H × 2, C-47,49), 119.9 (*C*H × 2, C-1,13), 117.6 (quat., C-22), 103.4 (*C*H, C-44), 86.3 (quat., C-26), 67.1 (CH_2, C-14), 55.0 (*C*H, C-34), 54.7 (*C*H, C-16), 53.5 (*C*H, C-52), 52.2 (CH_3, C-56), 47.0 (*C*H, C-7), 43.5 (CH_2, C-25), 39.9 (CH_2, 17), 36.8 (CH_2, C-35), 31.0 (*C*H, C-53), 29.1 (CH_2, C-19), 28.5 (quat. × 2, C-27), 25.5 (CH_2, C-18), 19.4 (CH_3, C-30), 19.0 (CH_3, C-32), 18.0 (CH_3 × 2, C-54) and 12.5 (CH_3, C-23); ***m/z*** (ES^+) 1112 ($[M+H]^+$, 100%); **HRMS** *m/z* (ES^+) calcd. for $C_{58}H_{65}N_9O_{12}S$ $[M+H]^+$ requires 1112.4546, found 1112.4546.

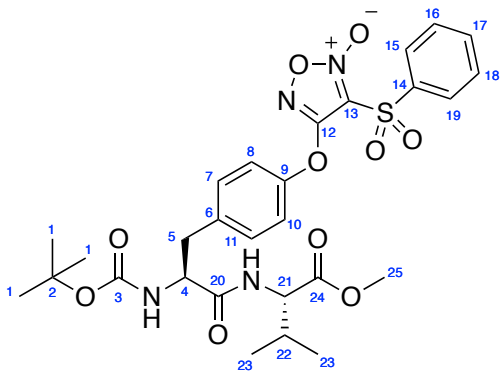
Fmoc-Asp(OtBu)-Tyr(*O*-carbonyl-*N*-3-phenylsydnonimine)-Phe-OMe, 332



HCl in dioxane (4M, 3 mL, 12.0 mmol) was added to a solution of dipeptide **330** (201 mg, 0.32 mmol) in dichloromethane (10 mL) cooled to 0 °C. The solution was stirred at 0 °C for 3 h and the solvent evaporated under reduced pressure to yield a white residue which was triturated with diethyl ether. The residue was dissolved in dry DMF (5 mL) and Fmoc-Asp(OtBu)-OH (132 mg, 0.32 mmol) was added. The solution was cooled to 0 °C and stirred for 10 min. HATU (442 mg, 1.297 mmol) was added and the solution stirred for a further 15 min. Diisopropylethylamine (distilled, 166 mg, 220 µL, 1.28 mmol) was added and the solution was allowed to warm to room temperature and stirred for 18 h. The resultant solution was diluted with ethyl acetate (50 mL) and water (50 mL). The organic layer was separated and washed with aq. hydrochloric acid (2 N, 20 mL), saturated $NaHCO_3$ (20 mL) and brine (20 mL). The organic layer was separated and dried over Na_2SO_4, filtered and the solvent removed under reduced pressure. The resultant residue was uptaken in dichloromethane and adsorbed onto silica gel. Purification by silica gel chromatography, eluting with dichloromethane and methanol (99:1) provided *Fmoc-Asp*(*OtBu)-Tyr*(*O-carbonyl-N-3-phenylsydnonimine)-Phe-OMe* **332** (250 mg, 0.27 mmol, 85%) as a white solid: $\boldsymbol{R_f}$ 0.59 (90:10, CH_2Cl_2:MeOH, UV/cerium phosphomolybdate); **m.p.** 116-117 °C; $[\alpha]_D^{20}$ +157, (*c* = 1.0 , $CHCl_3$); ν_{max} (NaCl/thin film)/cm^{-1} 1734, 1670, 1603, 1588, 1513, 1495, 1451, 1368, 1216, 1050: **^{1}H NMR** (500 MHz, $CDCl_3$) δ 8.20 (1H, s, C*H*-31), 7.82 (2H, d, *J* 7.9 Hz, C*H*-33,37), 7.75-7.67 (3H, m, C*H*-2,12,35), 7.63-7.52 (4H, m, C*H*-5,9,34,36), 7.38-7.20 (7H, m, C*H*-3,4,10,11,43,44,45), 7.09-7.05 (4H, m, C*H*-25,29,43,47), 6.91 (2H, d, *J* 8.1 Hz, C*H*-26,28), 6.74 (1H, d, *J* 8.1, N*H*-amide), 6.60 (1H, d, *J* 7.6, N*H*-amide), 5.96 (1H, d, *J* 8.5, N*H*-Fmoc), 4.77 (1H, dd, *J* 13.4, 6.7 Hz, C*H*-40), 4.64 (1H, dd, *J* 15.0, 7.9 Hz, C*H*-22), 4.47-4.40 (3H, m, C*H*-16, CH_2-14), 4.19

(1H, t, *J* 6.5 Hz, C*H*-7), 3.67 (3H, s, CH_3-49), 3.15-3.08 (2H, m, CH_AH$_B$-23, CH_AH$_B$-41), 3.00 (1H, dd, *J* 13.8, 6.8 Hz, CH$_A$$H_B$-41), 2.91 (1H, dd, *J* 14.2, 8.2 Hz, CH$_A$$H_B$-23), 2.62 (1H, dd, *J* 16.5, 5.6 Hz, CH_AH$_B$-16), 2.56 (1H, dd, *J* 16.5, 7.2 Hz, CH$_A$$H_B$-16) and 1.39 (9H, s, 3 × CH_3-19); **^{13}C NMR** (125 MHz, $CDCl_3$) 175.8 (quat., C-31), 171.5 (quat., C-48), 170.8 (quat., C-39), 170.8 (quat., C-21), 170.1 (quat., C-18), 160.6 (quat., C-30), 156.4 (quat., C-15), 151.0 (quat., C-27), 143.8, 143.7 (*C*H × 2, C-6,8), 141.3 (quat. × 2, C-1,13), 135.8 (quat., C-42), 133.7 (quat., C-33), 133.4 (*C*H, C-36), 133.4 (quat., C-24), 130.5 (*C*H × 2, C-34, 38), 130.1 (*C*H × 2, C-25, 29), 129.2 (*C*H × 2, C-43,47), 128.6 (*C*H × 2, C-44,46), 127.8, 127.5 (*C*H × 2, C-4,10), 127.1 (*C*H × 2, C-3,11), 127.0 (*C*H, C-45), 125.0, 124.9 (*C*H × 2, C-5,9), 122.2 (*C*H × 2, C-26,28), 121.6 (*C*H × 2, C-35,37), 120.1, 120.0 (*C*H × 2, C-2,13), 103.6 (*C*H, C-32), 81.6 (quat., C-19), 67.0 (*C*H$_2$, C-14), 54.0 (*C*H, C-22), 53.4 (*C*H, C-40), 52.4 (*C*H$_3$, C-49), 51.6 (*C*H, C-16), 47.1 (*C*H, C-7), 37.8 (*C*H$_2$, C-41), 37.2 (*C*H$_2$, C-17), 36.8 (*C*H$_2$, C-23) and 28.0 (*C*H$_3$ × 3, C-20); ***m/z*** (ES$^+$) 923 ([M+H]$^+$, 100%); **HRMS** *m/z* (ES$^+$) calcd. for $C_{51}H_{50}N_6O_{11}$ [M+H]$^+$ requires 923.3610, found 923.3614.

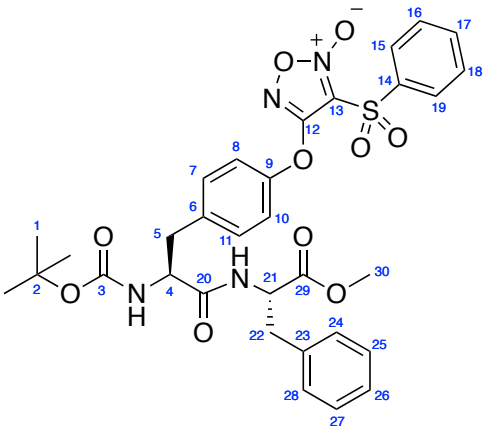
Boc-Tyr((*O*-(3-(phenylsulfonyl)-1,2,5-oxadiazole 2-oxide))-Val-OMe, 333



HATU (113 mg, 0.30 mmol) was added to a solution of L-valine methyl ester hydrochloride (18 mg, 0.11 mmol) and (*S*)-4-(4-(2-((*tert*-butoxycarbonyl)amino)-2-carboxyethyl)phenoxy)-3-(phenylsulfonyl)-1,2,5-oxadiazole 2-oxide **312** (50 mg, 0.11 mmol) in dry DMF (5 mL) cooled to 0 °C. The solution was stirred for 15 min and diisopropylethylamine (distilled, 51 mg, 70 µL, 0.40 mmol) was added. The solution was allowed to warm to room temperature and stirred for 18 h. The resultant solution was diluted with ethyl acetate (50 mL) and water (50 mL). The organic layer was separated and washed with aq. hydrochloric acid (2 N, 20 mL), saturated $NaHCO_3$ (20 mL) and brine (20 mL). The organic layer was separated and dried over Na_2SO_4, filtered and the solvent removed under reduced pressure. The resultant residue was uptaken in dichloromethane and adsorbed onto silica gel. Purification by silica gel chromatography, eluting with ethyl acetate and hexanes (10:90 to 25:75) provided *Boc-Tyr((O-(3-(phenylsulfonyl)*-1,2*,5-oxadiazole 2-oxide))-Val-OMe* **333** (55 mg, 0.09 mmol, 95%) as a white solid: ***R$_f$*** 0.60 (90:10,

CH_2Cl_2:MeOH); **m.p.** 126-128 °C; $[\alpha]_D^{20}$-9.2, (*c* = 0.1, $CHCl_3$); ν_{max} (NaCl/thin film)/cm^{-1} 3019, 2927, 1741, 1699, 1678, 1618, 1533, 1505, 1449, 1438, 1368, 1215, 1168, 1018**:** ^{1}**H NMR** (500 MHz, $CDCl_3$) δ 8.15-8.09 (2H, d, *J* 8.3 Hz, C*H*-15,19), 7.78 (1H, t, *J* 7.6 Hz, C*H*-17), 7.67-7.63 (2H, m, C*H*-16,18), 7.30-7.26 (2H, m, C*H*-7, 11), 7.23-7.19 (2H, m, C*H*-8,10), 6.45-6.36 (1H, m, N*H*-amide), 5.05 (1H, s, *br*, N*H*-Boc), 4.46 (1H, dd, *J* 8.8, 5.0 Hz, C*H*-21), 4.38-4.33 (1H, m, C*H*-4), 3.71-3.68 (3H, m, CH_3-25), 3.15-3.10 (1H, m, CH_AH_B-5), 3.08-3.02 (1H, m, CH_AH_B-5), 2.16-2.07 (1H, m, C*H*-22), 1.42 (9H, s, 3 × *CH*$_3$-1), 0.89 (3H, d, *J* 6.9 Hz, CH_3-23) and 0.86 (3H, d, *J* 6.9 Hz, CH_3-23); ^{13}**C NMR** (125 MHz, CD_3CN) δ 171.8 (quat., C-24), 170.8 (quat., C-20), 158.3, 160.6 (quat., C-12), 155.5 (quat., C-3), 151.7, 152.8 (quat., × 2, C-9), 137.9, 137.8 (quat., C-14), 135.8, 135.6 (*C*H, C-17), 135.5, 135.3 (quat., C-6), 130.9 (*C*H × 2, C-7, 11), 129.0 (*C*H × 2, C-16,18) 128.6 (*C*H × 2, C-15,19), 119.9, 119.3 (*C*H × 2, C-8,10), 110.8 (quat., C-13), 80.5 (quat., C-2), 57.3 (*C*H, C-21), 55.7 (*C*H, C-4), 52.2 (*C*H_3, C-25), 37.2 (*C*H_2, C-5), 31.2 (*C*H, C-22), 28.3 (*C*H_3 × 3, C-1) and 18.6, 17.7 (*C*H_3 × 2, C-23); ***m/z*** (ES^+) 641 ($[M+Na]^+$, 100%); **HRMS** *m/z* (ES^+) calcd. for $C_{28}H_{34}N_4NaO_{10}S$ $[M+Na]^+$ requires 641.1893, found 641.1900.

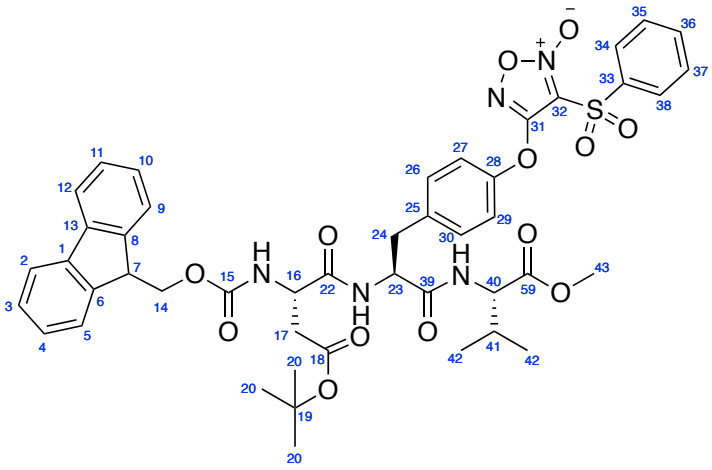
Boc-Tyr((*O*-(3-(phenylsulfonyl)-1,2,5-oxadiazole 2-oxide))-Phe-OMe, 334



HATU (113 mg, 0.30 mmol) was added to a solution of phenylalanine methyl ester hydrochloride (872 mg, 1.66 mmol) and (*S*)-4-(4-(2-((*tert*-butoxycarbonyl)amino)-2-carboxyethyl)phenoxy)-3-(phenylsulfonyl)-1,2,5-oxadiazole 2-oxide **312** (50 mg, 0.10 mmol) in dry DMF (5 mL) cooled to 0 °C. The solution was stirred for 15 min and diisopropylethylamine (distilled, 51 mg, 70 µL, 0.40 mmol) was added. The solution was allowed to warm to room temperature and stirred for 18 h. The resultant solution was diluted with ethyl acetate (20 mL) and water (20 mL). The organic layer was separated and washed with aq. hydrochloric acid (2 N, 20 mL), saturated $NaHCO_3$ (20 mL) and brine (20 mL). The organic layer was separated and dried over Na_2SO_4, filtered and the solvent removed under reduced pressure. The resultant residue was uptaken in dichloromethane and adsorbed onto silica gel. Purification by silica gel

chromatography, eluting with ethyl acetate and hexanes (10:90 to 25:75) provided *Boc-Tyr((O-(3-(phenylsulfonyl)*-1,2*,5-oxadiazole 2-oxide))-Phe-OMe* **334** (60 mg, 0.09 mmol, 95%) as a white solid: R_f 0.62 (90:10, CH_2Cl_2:MeOH); **m.p.** 118-121 °C; $[\alpha]_D^{20}$ -22.0, (*c* =0.1 , $CHCl_3$); ν_{max} (NaCl/thin film)/cm^{-1} 2960, 2924, 2853, 1741, 1658, 1617, 1535, 1505, 1450, 1366, 1259, 1202, 1167, 1019; **^{1}H NMR** (500 M, $CHCl_3$) δ 8.18-8.12 (2H, m, C*H*-15,19), 7.81 (1H, tt, *J* 7.5, 1.2 Hz, C*H*-17), 7.67 (2H, t, *J* 8.0 Hz, C*H*-16,18), 7.28-7.20 (7H, m, C*H*-7,8,10,11,25,26,27), 7.05 (2H, d, *J* 7.4 Hz, C*H*-24,28), 6.34 (1H, d, *J* 7.4, N*H*-amide), 4.99 (1H, s, *br*, N*H*-Boc), 4.81 (1H, dd, *J* 13.3, 6.4 Hz, C*H*-21), 4.37-4.33 (1H, m, C*H*-4), 3.72 (3H, s, CH_3-30), 3.14-3.02 (4H, m, CH_2-5, 22) and 1.43 (9H, s, 3 × CH_3-1); **^{13}C NMR** (125 M, $CHCl_3$) δ 171.4 (quat., C-29), 170.5 (quat., C-20), 158.4 (quat., C-12), 155.3 (quat., C-3), 151.6 (quat., C-9), 138.0 (quat., C-14), 135.9 (*C*H, C-17), 135.5 (quat., C-6), 135.3 (quat., C-23), 130.9 (*C*H × 2, C-7,11), 129.8 (*C*H, C-26), 129.2 (*C*H × 2, C-16,18), 128.6 (*C*H × 2, C-15,19), 128.6 (*C*H × 2, C-24,28), 127.2 (*C*H × 2, C-25,27), 120.0 (*C*H × 2, C-8,10); 110.7 (quat., C-13), 80.3 (quat., C-2), 53.3 (*C*H, C-21), 52.4 (CH_3, C-30), 51.6 (*C*H, C-4), 37.9 (CH_2, C-22), 37.6 (CH_2, C-5) and 28.1 (CH_3 × 3, C-1); ***m/z*** (ES^+) 667 ($[M+H]^+$, 100%); **HRMS** *m/z* (ES^+) calcd. for $C_{32}H_{35}N_4O_{10}S$ $[M+H]^+$ requires 667.2074, found 667.2078.

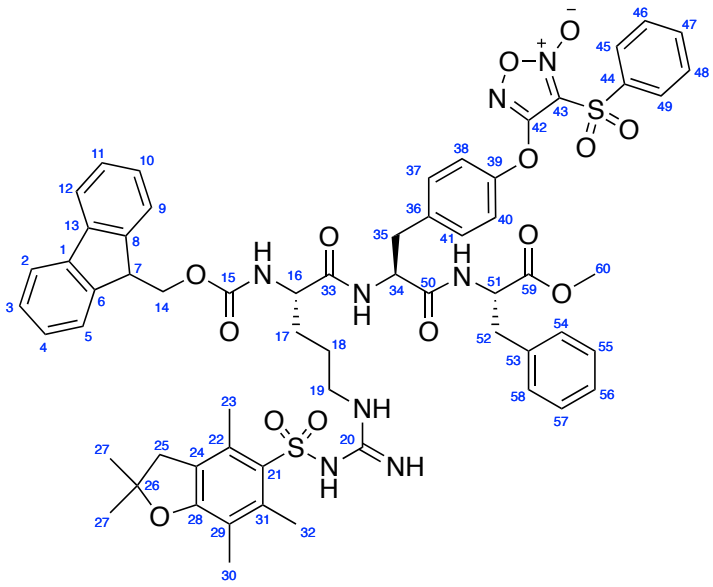
Fmoc-Asp(OtBu)-Tyr((*O*-(3-(phenylsulfonyl)-1,2,5-oxadiazole 2-oxide))-Val-OMe, 335



HCl in dioxane (4M, 1 mL, 4.0 mmol) was added to a solution of dipeptide **333** (62 mg, 0.10 mmol) in dichloromethane (10 mL) cooled to 0 °C. The solution was stirred at 0 °C for 3 h and the solvent evaporated under reduced pressure to yield a white residue which was triturated with diethyl ether. The residue was dissolved in dry DMF (5 mL) and Fmoc-Asp(OtBu)-OH (41 mg, 0.10 mmol) was added. The solution was cooled to 0 °C and stirred for 10 min. HATU (113 mg, 0.30 mmol) was added and the solution stirred for a further 15 min. Diisopropylethylamine (distilled, 39 mg, 50 μL, 0.30 mmol) was added and the solution was allowed to warm to room

temperature and stirred for 18 h. The resultant solution was diluted with ethyl acetate (50 mL) and water (50 mL). The organic layer was separated and washed with aq. hydrochloric acid (2 N, 20 mL), saturated $NaHCO_3$ (20 mL) and brine (20 mL). The organic layer was separated and dried over Na_2SO_4, filtered and the solvent removed under reduced pressure. The resultant residue was uptaken in dichloromethane and adsorbed onto silica gel. Purification by silica gel chromatography, eluting with ethyl acetate and hexanes (10:90 to 25:75) provided *Fmoc-Asp*(*OtBu)-Tyr((O-(3-(phenylsulfonyl)*-1,2*,5-oxadiazole 2-oxide))-Val-OMe* **335** (72 mg, 0.08 mmol, 80%) as a white solid: $\boldsymbol{R_f}$ 0.65 (90:10, CH_2Cl_2:MeOH, UV/cerium phosphomolybdate); **m.p.** 135-139 °C (decomp.); $[\boldsymbol{\alpha}]_{\mathbf{D}}^{\mathbf{20}}$ -22.0, (*c* = 0.1, $CHCl_3$); $\boldsymbol{\nu_{max}}$ (NaCl/thin film)/cm^{-1} 1733, 1700, 1656, 1648, 1581, 1553, 1539, 1494, 1450, 1404, 1367, 1260, 1181; **^{1}H NMR** (500 MHz, $CDCl_3$) δ 8.17-8.08 (2H, m, C*H*-33,37), 7.81-7.44 (3H, m, C*H*-5,9,35), 7.63 (2H, t, *J* 7.6 Hz, C*H*-34,36), 7.60-7.55 (2H, m, C*H*-4,10), 7.43-7.37 (2H, m, C*H*-2,12), 7.35-7.25 (4H, m, C*H*-3,11,25, 29), 7.13-7.10 (2H, m, C*H*-26,28), 7.02-6.99 (1H, m, N*H*-amide), 6.43 (1H, s, *br*, N*H*-amide), 5.78 (1H, dd, *J* 19.0, 8.3 N*H*-Fmoc), 4.64 (1H, t, *J* 6.7 Hz, C*H*-22), 4.49-4.34 (4H, m, C*H*-16, 39, CH_2-14), 4.20 (1H, t, *J* 6.7 Hz, C*H*-7), 3.78-3.71 (3H, m, CH_3-43), 3.17-3.04 (2H, m, CH_2-23), 2.85-2.80 (1H, m, CH_AH$_B$-17), 2.61 (1H, dd, *J* 17.0, 5.8 Hz, CH$_A$*H*$_B$-17), 2.14-2.08 (1H, m, C*H*-40), 1.41 (9H, s, 3 × *CH*$_3$-20) and 0.86-0.83 (6H, m, 2 × *CH*$_3$-41); **^{13}C NMR** (125 MHz, $CDCl_3$) δ 171.8 (quat., C-42), 171.0 (quat., C-38), 170.7 (quat., C-21), 169.9 (quat., C-18), 160.7, 158.4 (quat., C-30) 156.1 (quat., C-15), 152.8, 151.4 (quat., C-27), 141.3 (quat., C-6,8), 141.6 (quat. × 2, C-1,13), 137.8, 137.0 (quat., C-32), 135.8, 135.6 (*C*H, C-35), 135.2, 135.1 (quat., C-24), 131.0 (*C*H × 2, C-25,29), 129.8 (*C*H × 2, C-34, 36), 129.1, 128.6 (*C*H × 2, C-33,37), 127.7 (*C*H × 2, C-3,11), 127.1 (*C*H × 2, C-4,10), 125.0 (*C*H × 2, C-5,9), 120.1 (*C*H × 2, C-26,18), 119.4 (*C*H × 2, C-2,12), 110.7 (quat., C-31), 82.1 (quat., C-19), 67.2 (*C*H$_2$, C-14), 57.4 (*C*H, C-16), 54.4 (*C*H, C-22), 52.2 (*C*H$_3$, C-43), 51.2 (*C*H, C-39), 47.1 (*C*H, C-7), 37.1 (*C*H$_2$, C-17), 36.9 (*C*H$_2$, C-23), 31.0 (*C*H, C-40) 28.0 (*C*H × 3, C-20) and 18.9, 17.9 (*C*H$_3$ × 2, C-41); ***m/z*** (ES^+) 913 ($[M+H]^+$, 100%); **HRMS** *m/z* (ES^+) calcd. for $C_{46}H_{50}N_5O_{13}S$ $[M+H]^+$ requires 912.3125, found 912.3126.

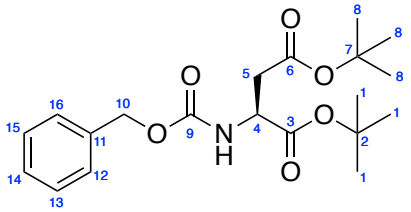
Fmoc-Arg(Pbf)-Tyr((*O*-(3-(phenylsulfonyl)-1,2,5-oxadiazole 2-oxide))-Phe-OMe, 336



HCl in dioxane (4M, 1 mL, 4.0 mmol) was added to a solution of dipeptide **334** (66 mg, 0.10 mmol) in dichloromethane (10 mL) cooled to 0 °C. The solution was stirred at 0 °C for 3 h and the solvent evaporated under reduced pressure to yield a white residue which was triturated with diethyl ether. The residue was dissolved in dry DMF (5 mL) and Fmoc-Arg(Pbf)-OH (64 mg, 0.10 mmol) was added. The solution was cooled to 0 °C and stirred for 10 min. HATU (113 mg, 0.30 mmol) was added and the solution stirred for a further 15 min. Diisopropylethylamine (distilled, 39 mg, 50 µL, 0.30 mmol) was added and the solution was allowed to warm to room temperature and stirred for 18 h. The resultant solution was diluted with ethyl acetate (50 mL) and water (50 mL). The organic layer was separated and washed with aq. hydrochloric acid (2 N, 20 mL), saturated $NaHCO_3$ (20 mL) and brine (20 mL). The organic layer was separated and dried over Na_2SO_4, filtered and the solvent removed under reduced pressure. The resultant residue was uptaken in dichloromethane and adsorbed onto silica gel. Purification by silica gel chromatography, eluting with ethyl acetate and hexanes (10:90 to 25:75) provided *Fmoc-Arg(Pbf)-Tyr((O-(3-(phenylsulfonyl)*-1,2*,5-oxadiazole 2-oxide))-Phe-OMe* **336** (103 mg, 0.09 mmol, 85%) as a white solid: $\boldsymbol{R_f}$ 0.62 (90:10, CH_2Cl_2:MeOH, UV/cerium phosphomolybdate); m**.p.** 109-110 °C; $[\alpha]_D^{20}$ -9.2, (*c* = 0.1 , $CHCl_3$); $\boldsymbol{\nu_{max}}$ (NaCl/thin film)/cm^{-1} 1752, 1730, 1635, 1600, 1540, 1491, 1450, 1270, 1175, 1040; **^{1}H NMR** (500 MHz, $CDCl_3$) δ 8.11-8.05 (2H, m, C*H*-45,49), 7.71-7.70 (3H, m, C*H*-5,9,47), 7.62-7.53 (4H, m, C*H*-4,10,46,48), 7.39-7.15 (8H, m, C*H*-2,3,11,12,54,55,58,57), 7.14-7.02 (5H, m, C*H*-30,37,38,41,56), 6.94 (1H, s, *br*, N*H*-amide), 6.36 (2H, s, *br*, N*H*-guanidine), 5.95 (1H, s, *br*, N*H*-Fmoc), 4.70 (1H, dd, *J* 6.7, 13.9 Hz, C*H*-51), 4.57 (1H, t, *J* 7.1 Hz, C*H*-34), 4.39-4.22 (3H, m, CH_2-14, C*H*-16), 4.11 (1H, t, *J*

7.4 Hz, C*H*-7), 3.60 (3H, s, CH_3-60), 3.17-2.85 (8H, m, CH_2-17,25,35,52), 2.61 (3H, s, CH_3-23), 2.51 (3H, s, CH_3-30), 2.07 (3H, s, C*H*-32), 1.82-1.74 (1H, m, CH_AH$_B$-19), 1.65-1.54 (1H, m, CH$_A$$H_B$-19) and 1.47-1.40 (8H, CH_2-18, 3 × CH_3-27); **^{13}C NMR** (125 MHz, $CDCl_3$) δ 172.6 (quat., C-33), 171.7 (quat., C-59), 170.9 (quat., C-50), 159.0 (quat., C-42), 158.3 (quat., C-28), 156.6 (quat., C-20), 156.4 (quat., C-15), 151.3 (quat, C-39), 143.8, 143.7 (quat. × 2, C-6,8), 141.2 (quat. × 2, C-1,13), 138.5 (quat., C-21), 138.0 (quat., C-44), 136.0 (quat., C-36), 135.8 (*C*H, C-47), 135.4 (quat., C-53), 132.4 (quat., C-24), 132.3 (quat., C-29), 130.8 (*C*H × 2, C-37, 41), 129.8 (*C*H × 2, C-55,57), 129.2 (*C*H × 2, C-45,49), 128.6 (*C*H × 2, C-46,48), 128.8 (*C*H, C-56), 128.7 (*C*H × 2, C-54,58), 127.7 (*C*H × 2, C-3,11), 127.1 (*C*H × 2, C-4,10), 125.2 (*C*H × 2, C-5,9), 124.8 (quat., C-31), 120.0 (*C*H × 2, C-38 40), 119.9 (*C*H × 2, C-2,12), 117.7 (quat., C-22), 110.7 (quat., C-43), 86.5 (quat., C-26), 67.1 (*C*H_2, C-14), 56.8 (*C*H, C-51), 53.7 (*C*H, C-16) 55.5 (*C*H, C-34), 52.4 (*C*H_3, C-60), 47.0 (*C*H, C-7), 43.2 (*C*H_2, C-17), 43.2 (*C*H_2, C-25), 37.7 (*C*H_2, C-35), 36.8 (*C*H_2, C-52). 30.3 (*C*H_2, C-19), 28.6 (quat. × 2, C-27), 25.2 (*C*H_2, C-18), 19.4 (*C*H_3, C-30), 18.1 (*C*H_3, C-32) and 12.5 (*C*H_3, C-23); ***m/z*** (ES^+) 1197 ($[M+H]^+$, 100%); **HRMS** *m/z* (ES^+) calcd. for $C_{61}H_{65}N_8O_{14}S$ $[M+H]^+$ requires 1197.4057, found 1197.4062.

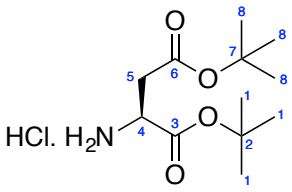
Cbz-Asp(OtBu)-OtBu, 340[377]



Boron trifluoride diethyl etherate (930 µL, 7.5 mmol) was added to a suspension of Cbz-Asp-OH **339** (10.0 g, 37.4 mmol) in *tert*-butyl acetate (55 mL), and the suspension stirred overnight. After ca. 3 h a clear solution was attained. The solution was quenched with water (25 mL), basified to pH 12 with aq. sodium hydroxide (4N) and extracted with ethyl acetate (3 × 50 mL). The ethyl acetate layer was separated and the solvent removed under reduced pressure to give Cbz-Asp(OtBu)-OtBu **340** (8.50 g, 22.4 mmol, 60%) as a colourless oil, which was used without any further purification: R_f 0.31 (90:10, EtOAc:PE, UV/$KMnO_4$); $[\alpha]_D^{20}$ +4.6, (*c* = 1.0, dioxane, [Lit.[418] +4.5 (*c* = 1.0, dioxane]; **^{1}H NMR** (500 MHz, $CDCl_3$) δ 7.36-7.28 (5H, m, C*H*-12,13,14,15,16), 5.75 (1H, d, *J* 8.7 Hz, N*H*-Cbz), 5.11 (2H, s, CH_2-10), 4.19 (1H, dt, *J* 8.7, 4.4 Hz, C*H*-4), 2.87 (1H, dd, *J* 17.1, 4.4 Hz, CH_AH$_B$-5), 2.70 (1H, dd, *J* 16.9, 4.3 Hz, C$H_A$$H_B$-5), 1.45-1.52 (18H, m, 3 × CH_3-1,3 × CH_3-8); **^{13}C NMR** (125 MHz, $CDCl_3$) δ 170.2, 169.9 (quat. × 2, C-3,6), 156.1 (quat., C-9), 136.4 (quat., C-11), 128.5 (*C*H × 2, C-15,13), 128.1 (*C*H, C-4), 128.0 (*C*H × 2, C-12,16), 82.3, 81.6 (quat. × 2, C-2,7), 66.9 (*C*H_2, C-10), 51.0 (*C*H, C-4), 37.9

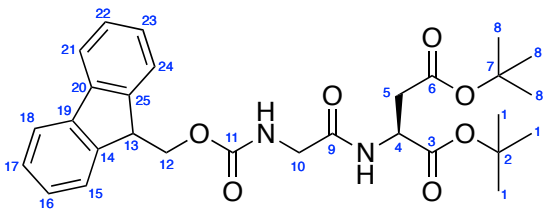
(CH_2, C-5), 28.0, 27.9 (2 × CH_3 × 3, C-1, C-8); ***m/z*** (ES^+) 402 ($[M+Na]^+$, 100%). The data were in agreement with the literature values.[377]

NH_2-Asp(OtBu)-OtBu hydrochloride, 338[377]



Pd/C (10%, 830 mg, 10% *w*/*w*) was added to a solution of Cbz-Asp(OtBu)-OtBu **340** (8.25 g, 21.8 mmol) and ammonium formate (34.3 g, 0.54 mol) in ethanol (20 mL) and the suspension stirred overnight at room temperature. The suspension was filtered through Celite and the solvent removed under reduced pressure. The resultant residue was uptaken in ethyl acetate and cooled to 0 °C and a saturated solution of HCl in ethyl acetate was added dropwise and the solution stirred for 15 min. The precipitated solid was filtered, washed with cold ethyl acetate and dried under vacuum to give NH_2-Asp(OtBu)-OtBu hydrochloride, **338** (4.28 g, 17.4 mmol, 80%) as a white solid, which was used without any further purification: **m.p.** 157-160 °C, [Lit.[419] 156-158 °C]; $[\alpha]_D^{20}$ +6.62, (*c* = 0.9, MeOH), [Lit.[419] +6.6 (*c* = 0.9, MeOH)]; **^{1}H NMR** (300 MHz, d_6-DMSO) δ 8.70 (3H, s, NH_2.*H*Cl), 4.07 (1H, t, *J* 5.4 Hz, C*H*-4), 2.89 (2H, d, *J* 5.4 Hz, CH_2-5), 1.41 (18H, s, 3 × CH_3-1, 3 × CH_3-8); **^{13}C NMR** (75 MHz, d6-DMSO) δ 168.9, 167.7 (quat., × 2, C-3,6), 83.2, 81.7 (quat., C-2, 7), 49.1 (*C*H, C-4), 35.5 (CH_2, C-5), 28.0, 27.8 (2 × CH_3 × 3, C-1,8); ***m/z*** (ES^+) 246 ($[M+H]^+$, 100%). The data were in agreement with the literature values. [377]

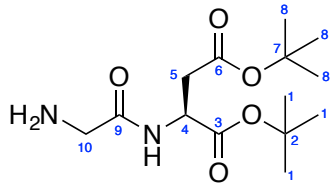
Fmoc-Gly-Asp(OtBu)-OtBu, 343[376]



Diisopropylamine (1.31 g, 1.76 mL, 10.1 mmol) was added to a solution of Fmoc-Gly-OH **341** (1.51 g, 5.05 mmol) and NH_2-Asp(OtBu)-OtBu.HCl **338** (1.43 g, 5.05 mmol) in dichloromethane (50 mL) cooled to 0 °C . T3P® (50 wt% in EtOAc, 3.65 mL, 6.2 mmol) was added *via* syringe pump over 30 min. The solution was allowed to warm to room temperature

and stirred for 24 h. Water (50 mL) was added, and the organic layer separated and washed with $NaHCO_3$ solution (saturated, 50 mL), aq. HCl (2 N, 50 mL) and brine (100 mL). The organic layer was dried over $MgSO_4$, filtered and the solvent removed under reduced pressure to give Fmoc-Gly-Asp(OtBu)-OtBu **343** (2.52 g, 4.80 mmol, 95%) as a white foam, which was used without any further purification: **m.p.** 57-59 °C, [Lit.[376] 55.4-60.2 °C]; $\boldsymbol{R_f}$ 0.58 (90:10, CH_2Cl_2:MeOH, UV/cerium phosphomolydate); $[\boldsymbol{\alpha}]_{\mathbf{D}}^{\mathbf{20}}$ +23.0, (*c* = 1.0, $CHCl_3$, [Lit.[376] +22.9 (*c* = 1.0, $CHCl_3$]; **^{1}H NMR** (500 MHz, $CDCl_3$) δ 7.75 (2H, d, *J* 7.6 Hz, C*H*-18,21), 7.60 (2H, d, *J* 6.9 Hz, C*H*-15,24), 7.38 (2H, t, *J* 7.5 Hz, C*H*-17,22), 7.30 (2H, t, *J* 7.6 Hz, C*H*-16,23), 6.96 (1H, s, *br*, N*H*-amide), 5.63 (1H, s, *br*, N*H*-Fmoc), 4.71 (1H, dt, *J* 8.4, 4.3 Hz, C*H*-4), 4.38 (2H, d, *J* 7.1 Hz, CH_2-12), 4.22 (1H, t, *J* 7.1 Hz, C*H*-13), 4.00-3.90 (2H, m, CH_2-10), 2.89 (1H, dd, *J* 17.2, 4.4 Hz, CH_AH$_B$-5), 2.72 (1H, dd, *J* 17.2, 4.1 Hz, CH_AH$_B$-5), 1.45-1.41 (18H, m, 3 × CH_3-1, 3 × CH_3-8); **^{13}C NMR** (125 MHz, $CDCl_3$) δ 171.2, 169.6, 168.7 (quat., C-3,6,9), 156.5 (quat., C-11), 143.8 (quat. × 2, C-14,25), 141.3 (quat. × 2, C-19,20), 127.7 (*C*H × 2, C-17,22), 127.1 *C*H × 2, C-16,23), 125.2 (*C*H × 2, C-15,24), 119.9 (*C*H × 2, C-18,21), 82.5, 81.7 (quat., C-2,7), 67.3 (*C*H_2, C-12), 49.1 (*C*H, C-4), 47.1 (*C*H, C-13), 44.3 (*C*H_2, C-10), 36.5 (*C*H_2, C-5), 28.0, 27.9 (*C*H_3 × 6, C-1, 8), 25.2 (*C*H_2, C-8); ***m/z*** (ES$^+$) 547 ([M+Na]$^+$, 100%). The data were in agreement with the literature values.[376]

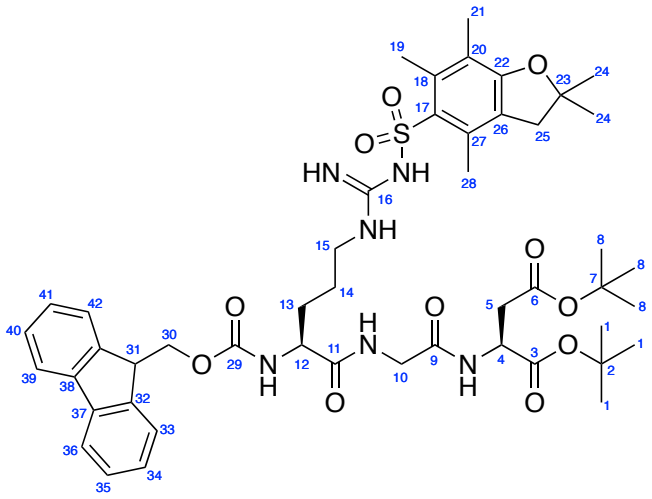
NH_2-Gly-Asp(OtBu)-OtBu, 344[376]



Piperidine (3 mL, 30.4 mmol) was added to a solution of Fmoc-Gly-Asp(OtBu)-OtBu **343** (1.70 g, 3.24 mmol) in dry DMF (10 mL) cooled in an ice-bath. After addition, the ice-bath was removed and the solution stirred for 15 min. The solvent was evaporated under reduced pressure to yield a yellow residue. The residue suspended in dichloromethane and adsorbed onto silica gel. Purification by silica gel chromatography eluting with dichloromethane and methanol (100:0 to 90:0) provided NH_2-Gly-Asp(OtBu)-OtBu **344** (855 mg, 2.83, 87%) as a colourless oil: $\boldsymbol{R_f}$ 0.25 (90:10, CH_2Cl_2:MeOH, ninhydrin); $[\boldsymbol{\alpha}]_{\mathbf{D}}^{\mathbf{20}}$ +36.5, (*c* = 1.0, $CHCl_3$, [Lit. +36.5 (*c* = 1.0, $CHCl_3$]; **^{1}H NMR** (500 MHz, $CDCl_3$) δ 7.98 (1H, d, *J* 8.4 Hz, N*H*-amide), 4.70 (1H, dt, *J* 8.8, 4.5 Hz, C*H*-4), 3.37 (2H, d, *J* 2.3 Hz, CH_2-10), 2.88 (1H, dd, *J* 4.4 Hz, *J* 16.8 Hz, CH_AH$_B$-5), 2.88 (1H, dd, *J* 4.5 Hz, *J* 16.9 Hz, CH_AH$_B$-5), 1.58 (2H, s, *br*, NH_2) and 1.44-1.42 (18H, m, 3 × CH_3-1, 3 × CH_3-8); **^{13}C NMR** (125 MHz, $CDCl_3$) δ 173.0 (quat., C-9), 170.3, 170.1 (quat. ×

2, C-3,6), 82.2, 81.8 (quat. × 2, C-2,7), 49.0 (*C*H, C-4), 45.0 (*C*H$_2$, C-10), 38.1 (C*H*$_2$, C-5) and 28.3, 28.2 (*C*H$_3$ × 6, C-1, 8); ***m/z*** (ES$^+$) 303 ([M+H]$^+$, 100%). The data were in agreement with the literature values. [376]

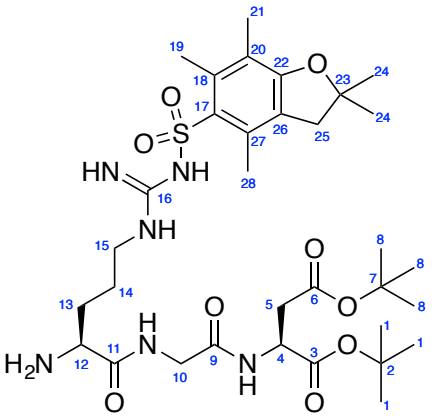
Fmoc-Arg(Pbf)-Gly-Asp(OtBu)-OtBu, 345[376]



Diisopropylamine (1.31 g, 1.76 mL, 10.1 mmol) was added to a solution of Fmoc-Arg(Pbf)-OH, (2.17 g, 3.34 mmol) and NH$_2$-Gly-Asp(OtBu)-OtBu **344** (1.01 g, 3.34 mmol) in dichloromethane (50 mL) cooled to 0 °C . T3P® (50 wt% in EtOAc, 2.50 mL, 4.25 mmol) was added *via* syringe pump over 30 min. The solution was allowed to warm to room temperature and stirred for 24 h. Water (50 mL) was added, and the organic layer separated and washed with aq. NaHCO$_3$ solution (saturated, 50 mL), aq. HCl (2 N, 50 mL) and brine (100 mL). The organic layer was dried over MgSO$_4$, filtered and the solvent removed under reduced pressure. The residue was uptaken in dichloromethane and adsorbed onto silica gel. Purification by silica gel chromatography eluting with dichloromethane and methanol (99:1 to 95:5) provided Fmoc-Arg(Pbf)-Gly-Asp(OtBu)-OtBu **345** (2.80 g 3.01 mmol, 90%) as a white foam: **m.p.** 116-119 °C, [Lit.[376] 114.0-120.0 °C]; R_f 0.55 (90:10, CH_2Cl_2:MeOH, UV/cerium phosphomolydate); $[\alpha]_D^{20}$ +8.6, (*c* = 1.0, $CHCl_3$, [Lit.[376] +8.6 (*c* = 1.0, $CHCl_3$]; **^{1}H NMR** (500 MHz, $CDCl_3$) δ 7.73 (2H, d, *J* 7.5 Hz, C*H*-36,39), 7.63 (1H, s, *br*, N*H*-Gly-Asp-amide), 7.58 (2H, d, *J* 6.3 Hz, C*H*-33,42), 7.36 (2H, t, *J* 7.4 Hz, C*H*-35,40), 7.26 (2H, t, *J* 7.4 Hz, C*H*-34,41), 7.02 (1H, d, *J* 6.3, N*H*-Arg-Gly-amide), 6.31 (2H, s, *br*, N*H*-guanidine × 2), 6.12 (1H, s, *br*, N*H*-sulfonamide), 6.01 (1H, d, *J* 5.7 Hz, N*H*-Fmoc), 4.64 (1H, dt, *J* 8.5, 4.3 Hz, C*H*-4), 4.40-4.33 (3H, m, C*H*-12, CH_2-30), 4.17 (1H, t, *J* 7.0 Hz, C*H*-7), 4.06 (1H, dd, *J* 17.3, 5.7 Hz, CH_AH$_B$-10), 3.91 (1H, dd, *J* 17.0, 5.7 Hz, CH_A*H*$_B$-10), 3.38-3.30 (1H, m, CH_AH$_B$-15), 3.24-3.17 (1H, m, CH_A*H*$_B$-15), 2.92 (2H, s, CH_2-29), 2.82 (1H, dd, *J* 17.1, 4.8 Hz, CH_AH$_B$-5), 2.67 (1H, dd, *J* 17.1, 4.4 Hz, CH_A*H*$_B$-

5), 2.59 (3H, s, CH_3-19), 2.51 (3H, s, CH_3-21), 2.07 (3H, s, CH_3-28), 1.76-1.68 (2H, m, CH_2-13), 1.64-1.54 (2H, m, CH_2-14), 1.43 (6H, s, 2 × CH_3-24), and 1.42-1.40 (18H, s, 3 × CH_3-1, 3 × CH_3-8); **^{13}C NMR** (125 MHz, CDCl3) δ 172.7, 170.2, 169.9, 169.2 (quat. × 4, C-3,6,9,12), 158.7 (quat., C-22), 156.6 (quat., C-16), 156.3 (quat., C-29), 143.9, 143.8 (quat. × 2, C-37, 38), 141.3 (quat. × 2, C-32,43), 138.4 (quat., C-17), 132.8 (quat., C-26), 132.4 (quat., C-20), 127.7 (*C*H × 2, C-35,40), 127.1 (*C*H × 2, C-34,41), 125.2 (*C*H × 2, C-33, 42), 124.6 (quat., C-18), 119.9 (*C*H × 2, C-36,39), 117.5 (quat., C-27), 86.4 (quat., C-23), 82.7, 81.8 (quat. × 2, C-2, C-7), 67.1 (*C*H_2, C-30), 54.0 (*C*H, C-12), 49.4 (*C*H, C-4), 47.1 (*C*H, C-31), 43.2 (*C*H_2, C-25), 42.8 (*C*H_2, C-10), 40.1 (*C*H_2, C-15), 37.3 (*C*H_2, C-5), 30.0 (*C*H_2, C-13), 28.6 (*C*H_3 × 2, C-24), 28.0, 27.9 (2 × *C*H_3 × 3, C-1,8), 25.0 (*C*H_2, C-14), 19.3 (*C*H_3, C-21), 18.0 (*C*H_3, C-19) and 12.5 (*C*H_3, C-28); ***m/z*** (ES$^+$) 933 ([M+H]$^+$, 100%). The data were in agreement with the literature values.[376]

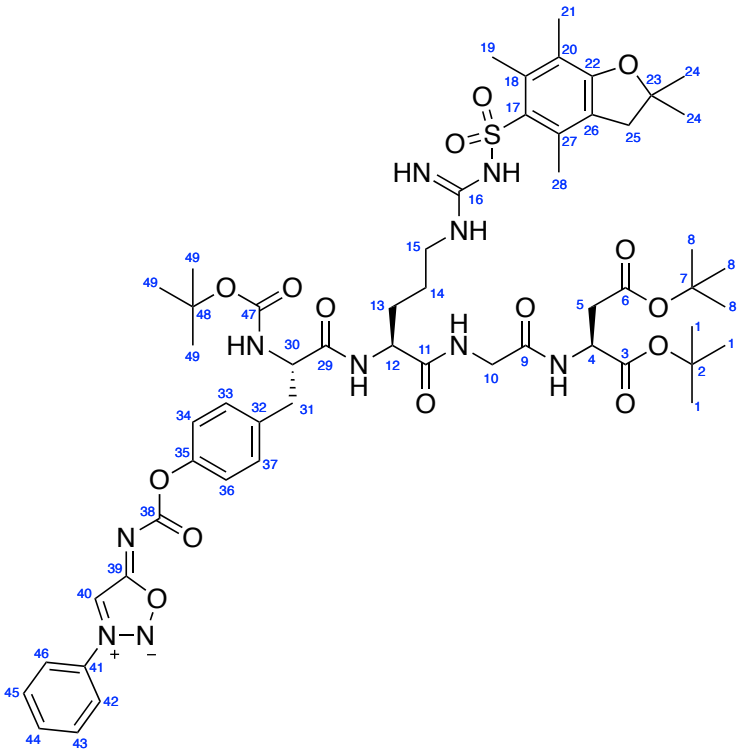
NH_2-Arg(Pbf)-Gly-Asp(OtBu)-OtBu, 341[376]



Piperidine (3.0 mL, 30.4 mmol) was added to a solution of Fmoc-Gly-Asp(OtBu)-OtBu, **345** (2.80 g, 3.00) in dry DMF (10 mL) cooled in an ice-bath. After addition, the ice-bath was removed and the solution stirred for 15 min. The solvent was evaporated under reduced pressure to yield a yellow residue. The residue suspended in dichloromethane and adsorbed onto silica gel. Purification by silica gel chromatography eluting with dichloromethane and methanol (100:0 to 90:0) provided NH_2-Arg(Pbf)-Gly-Asp(OtBu)-OtBu **341** (1.81 g, 2.55 mmol, 85%) as a colourless solid: **m.p.** 81-83°C, [Lit.[376] 83.0-84.0 °C]; ***R**$_f$* 0.33 (90:10, CH_2Cl_2:MeOH, UV/cerium phosphomolydate); **[α]$_D^{20}$** +18.1, (*c* = 1.0, $CHCl_3$, [Lit.[376] +18.2 (*c* = 1.0, $CHCl_3$]; **^{1}H NMR** (500 MHz, $CDCl_3$) δ 8.10 (1H, t, *J* 6.5, N*H*-Gly-Arg amide), 7.28 (1H, d, *J* 7.7, N*H*-Asp-Gly-amide), 6.46-6.33 (3H, m, N*H*-guanidine × 3), 4.65 (1H, dt, *J* 8.7, 4.6 Hz, C*H*-4), 4.00 (1H, dd, *J* 16.8, 5.1 Hz, CH_AH_B-10), 3.94 (1H, dd, *J* 16.8, 5.1 Hz, CH_AH_B-10), 3.47 (1H, t, *J* 6.9 Hz, C*H*-12), 3.24-3.19 (2H, m, CH_2-15), 2.95 (2H, s, CH_2-25), 2.83 (1H, dd, *J* 17.2, 4.9 Hz,

CH_AH$_B$-5), 2.70 (1H, dd, *J* 17.2, 4.4 Hz, C$H_A$$H_B$-5), 2.57 (3H, s, C$H_3$-19), 2.49 (3H, s, C$H_3$-21), 2.17-2.05 (5H, m, CH_3-28, NH_2), 1.84-1.78 (1H, m, CH_AH$_B$-13), 1.65-1.55 (3H, m, C$H_A$$H_B$-13, C$H_2$-14), 1.45 (6H, s, 9H, s, 2 × CH_3-24) and 1.42-1.41 (18H, s, 3 × CH_3-1, 3 × CH_3-8); **^{13}C NMR** (125 MHz, CDCl3) δ 172.1 (quat., C-11), 170.2 (quat., C-6), 169.7 (quat., C-3), 169.3 (quat., C-9), 158.6 (quat., C-22), 156.5 (quat., C-16), 138.2 (quat., C-17), 133.0 (quat., C-26), 132.2 (quat., C-20), 124.6 (quat., C-18), 117.4 (quat., C-27), 86.3 (quat., C-23), 82.5, 81.6 (quat. × 2, C-2, C-7), 54.4 (*C*H, C-12), 49.2 (*C*H, C-4), 43.2 (*C*H$_2$, C-25), 42.7 (*C*H$_2$, C-10), 40.5 (*C*H$_2$, C-15), 37.3 (*C*H$_2$, C-5), 31.9 (*C*H$_2$, C-13), 28.6 (*C*H$_3$ × 2, C-24), 28.0, 27.9 (2 × *C*H$_3$ × 3, C-1, C-8), 25.4 (*C*H$_2$, C-14), 19.3 (*C*H$_3$, C-21), 18.0 (*C*H$_3$, C-19) and 12.5 (*C*H$_3$, C-28); ***m/z*** (ES$^+$) 711 ([M+H]$^+$, 100%). The data were in agreement with the literature values.[376]

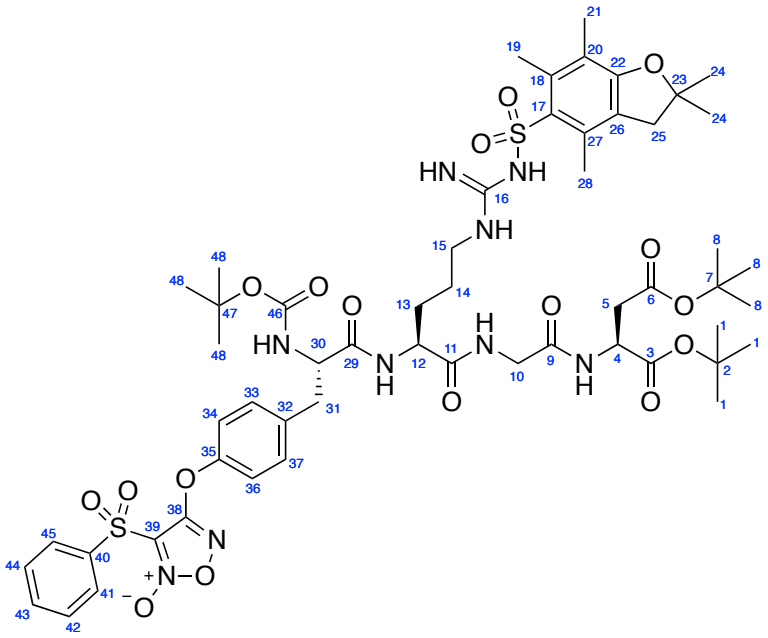
Boc-Tyr(*O*-carbonyl-*N*-3-phenylsydnonimine)-Arg(Pbf)-Gly-Asp(OtBu)-OtBu, 346



Following general procedure B, with HATU (217 mg, 0.572 mmol), NH$_2$-Arg(Pbf)-Gly-Asp(OtBu)-OtBu **341** (203 mg, 0.286 mmol), (*S*)-*N*-((4-(2-((tert-butoxycarbonyl)amino)-2-carboxyethyl)phenoxy)carbonyl)-3-phenylsydnonimine **308** (134 mg, 0.286 mmol) and diisopropylethylamine (distilled, 113 mg, 150 μL, 0.875 mmol). Purification by silica gel chromatography, eluting with dichloromethane and methanol (100:0 to 97:3) provided *Boc-Tyr(O-carbonyl-N-3-phenylsydnonimine)-Arg(Pbf)-Gly-Asp(OtBu)-OtBu* **346** (233 mg, 0.201 mmol, 70%) as a pale yellow solid: ***R$_f$*** 0.65 (90:10, CH_2Cl_2:MeOH, UV/cerium

phosphomolydate); **m.p.** 138-141 °C; $[\alpha]_D^{20}$ +13.0, (*c* = 0.2, $CHCl_3$); ν_{max} (thin film)/cm^{-1} 1734, 1684, 1652, 1588, 1558, 1537, 1506, 1455, 1393, 1369, 1108, 973, 847; **^{1}H NMR** (500 MHz, $CDCl_3$) δ 8.31 (1H, s, C*H*-40), 7.86 (2H, d, *J* 8.0 Hz, C*H*-42,46), 7.69-7.60 (4H, m, C*H*-43,44,45, N*H*-Arg-Gly-amide), 7.35 (1H, s, *br*, N*H*-Gly-Asp-amide), 7.19 (2H, d, *J* 7.7 Hz, C*H*-33,37), 7.06 (2H, d, *J* 8.0 Hz, C*H*-34,46), 6.40 (2H, s, *br*, N*H*-guanidine), 6.09 (1H, s, *br*, N*H*-sulfonamide), 5.65 (1H, s, *br*, N*H*-Boc), 4.66 (1H, dd, 3H = 5.0, 13.6 Hz, C*H*-4), 4.46-4.36 (2H, m, C*H*-12, 30), 3.95-3.89 (2H, m, CH_2-10), 3.21-3.13 (2H, m, CH_2-15), 3.11-3.06 (1H, m, CH_AH$_B$-31), 3.00-2.95 (1H, m, C$H_A$$H_B$-31), 2.91 (2H, s, C$H_2$-25), 2.79 (1H, dd, *J* 16.9, 5.4 Hz, CH_AH$_B$-5), 2.69 (1H, dd, *J* 17.2, 5.1 Hz, C$H_A$$H_B$-5), 2.53 (3H, s, C$H_3$-19), 2.46 (3H, s, C$H_3$-21), 2.04 (3H, s, C$H_3$-28), 1.90-1.82 (1H, m, CH_AH$_B$-13), 1.71-1.62 (1H, m, C$H_A$$H_B$-13), 1.49-1.30 (35H, m, 3 × CH_3-1, 3 × CH_3-8 Hz, CH_2-14, 2 × CH_3-24, 3 × CH_3-49); **^{13}C NMR** (125 MHz, $CDCl_3$) δ 175.5 (quat., C-39), 172.5, 172.2, 170.0, 169.8, 169.2 (quat. × 5, C-3,6,9,11,29), 159.9 (quat., C-38) 158.7 (quat., C-22) 156.4 (quat., C-16), 156.0 (quat., C-47), 150.8 (quat., C-35), 138.4 (quat., C-17), 133.6 (quat., C-41), 133.2 (*C*H, C-44), 133.7 (quat., C-32), 132.9 (quat., C-26), 132.3 (quat., C-20), 130.6 (*C*H × 2, C-42,46), 130.2 (*C*H × 2, C-33,37), 124.6 (quat., C-18), 121.9 (*C*H × 3, C-34,36), 121.7 (*C*H × 2, C-43,45), 117.5 (quat., C-27), 103.9 (*C*H, C-40), 86.4 (quat., C-23), 82.9 (quat., C-7), 81.5 (quat., C-2), 80.2 (quat., C-48), 53.2 (*C*H, C-12) 56.1 (*C*H, C-30), 49.4 (*C*H, C-4), 43.2 (*C*H_2, C-25), 42.8 (*C*H_2, C-10), 40.4 (*C*H_2, C-15), 37.5 (*C*H_2 × 2, C-5,31), 29.0 (*C*H_2, C-13), 28.6 (*C*H_3 × 2, C-24), 28.3 (*C*H_3 × 3, C-49), 28.0 (*C*H_3 × 3, C-8) 27.9 (*C*H_3 × 3, C-1), 25.1 (*C*H_2, C-14), 19.3 (quat., C-21), 18.0 (*C*H_3, C-19), 12.5 (*C*H_3, C-28); ***m/z*** (ES^+) 1161 ($[M+H]^+$, 100%); **HRMS** *m/z* (ES^+) calcd. for $C_{56}H_{76}N_{10}O_{15}S$ $[M+H]^+$ requires 1161.5286, found 1161.5266.

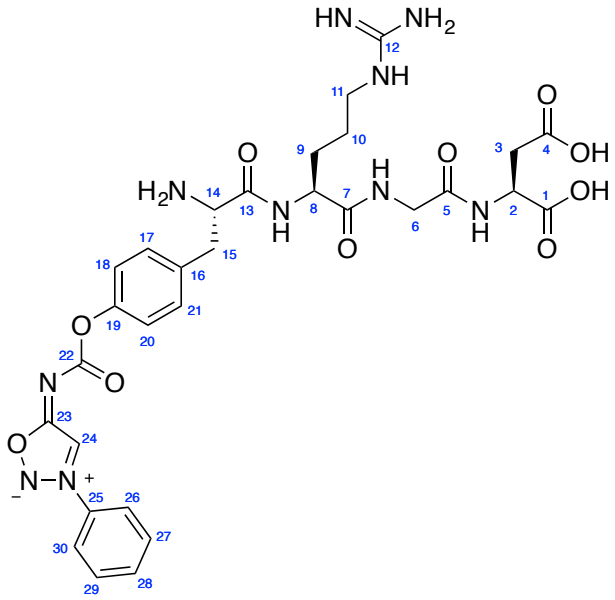
Boc-Tyr((*O*-(3-(phenylsulfonyl)-1,2,5-oxadiazole-2-oxide))-Arg(Pbf)-Gly-Asp(OtBu)-OtBu, 347



Following general procedure B, with HATU (221 mg, 0.58), NH_2-Arg(Pbf)-Gly-Asp(OtBu)-OtBu **341** (207 mg, 0.29 mmol), (*S*)-4-(4-(2-((*tert*-butoxycarbonyl)amino)-2-carboxyethyl)phenoxy)-3-(phenylsulfonyl)-1,2,5-oxadiazole 2-oxide **312** (147 mg, 0.29 mmol) and diisopropylethylamine (distilled, 113 mg, 150 µL, 0.88 mmol). Purification by silica gel chromatography, eluting with dichloromethane and methanol (100:0 to 97:3) provided *Boc-Tyr((O-(3-(phenylsulfonyl)-1,2,5-oxadiazole-2-oxide))-Arg(Pbf)-Gly-Asp(OtBu)-OtBu* **347** (241 mg, 0.20 mmol, 69%) as a white solid: $\boldsymbol{R_f}$ 0.69 (90:10, CH_2Cl_2:MeOH, UV/cerium phosphomolydate); **m.p.** 98-103 °C; $[\boldsymbol{\alpha}]_{\mathbf{D}}^{\mathbf{20}}$ +8.6, (*c* = 0.2, $CHCl_3$); $\boldsymbol{\nu_{max}}$ (thin film)/cm^{-1} 1905, 1734, 1700, 1683, 1654, 1639, 1498, 1456, 1103, 1020, 1051, 993, 970; **^{1}H NMR** (500 MHz, $CDCl_3$) δ 8.08 (2H, d, *J* 7.7 Hz, C*H*-45,41), 7.79-7.74 (2H, m, C*H*-43, N*H*-Arg-Gly-amide), 7.63 (2H, t, *J* 7.6 Hz, C*H*-42,44), 7.34 (1H, d, *J* 7.7, N*H*-Gly-Asp-amide), 7.28 2H, d, *J* 8.3 Hz, C*H*-33,37), 7.15 (2H, d, *J* 8.3 Hz, C*H*-34,36), 6.38 (2H, s, *br*, 2 × N*H*-guanidine), 6.10 (1H, s, *br*, N*H*-sulfonamide), 5.75 (1H, s, *br*, N*H*-Boc), 4.68-4.64 (1H, m, C*H*-4), 4.56-4.50 (1H, m, C*H*-12), 4.50-4.44 (1H, m, C*H*-30), 4.04-3.98 (2H, m, CH_2-10), 3.34-3.13 (3H, m, CH_2-15, CH_AH_B-31), 2.98-2.91 (3H, m, CH_2-25, CH_AH_B-31), 2.82 (1H, dd, *J* 17.1, 5.5 Hz, CH_AH_B-5), 2.69 (1H, dd, *J* 17.1, 5.0 Hz, CH_AH_B-5), 2.56 (3H, s, CH_3-19), 2.49 (3H, s, CH_3-21), 2.07 (3H, s, CH_3-28), 1.94-1.86 (1H, m, CH_AH_B-13), 1.76-1.67 (1H, m, CH_AH_B-13), 1.64-1.55 (2H, m, CH_2-14) and 1.44-1.32 (33H, m, 3 × CH_3-1, 3 × CH_3-8, 2 × CH_3-24, 3 × CH_3-48); **^{13}C NMR** (125 MHz, $CDCl_3$) δ 172.2, 170.0, 169.9, 169.3 (quat. × 5, C-3,6,9,11,29), 158.8 (quat., C-35), 158.5 (quat., C-39), 156.5 (quat., C-16), 156.0 (quat., C-46), 138.8 (quat., C-22), 138.5 (quat., C-17), 137.9 (quat., C-40), 136.0 (quat., C-32), 135.9 (*C*H, C-43), 132.7 (quat., C-26), 132.3

(quat., C-20), 131.0 (*C*H × 2, C-33,37), 129.8 (*C*H × 2, C-41,45), 128.6 (*C*H × 2, C-42,44), 124.7 (quat., C-18), 119.8 (*C*H × 2, C-34,36), 117.6 (quat., C-27), 110.8 (quat., C-38), 86.5 (quat., C-23), 82.5, 81.6 (quat. × 2, C-2,7), 80.1 (quat., C-47), 56.1 (*C*H, C-30), 53.1 (*C*H, C-12), 49.5 (*C*H, C-4), 43.3 (CH_2, C-25), 42.7 (C-10), 40.5 (CH_2, C-15), 37.8 (CH_2, C-31), 37.4 (CH_2, C-5), 29.3 (CH_2, C-13), 28.6 (CH_3 × 2, C-24), 28.3 (CH_3 × 3, C-48), 28.0 and 27.9 (2 × CH3 × 3, C-1,8), 25.1 (CH_2, C-14), 19.4 (CH_3, C-21), 18.0 (CH_3, C-19), 12.5 (CH_3, C-28); ***m/z*** (ES^+) 1198 ($[M+H]^+$, 100%); **HRMS** *m/z* (ES^+) calcd. for $C_{55}H_{75}N_9O_{17}S_2$ $[M+H]^+$ requires 1198.4795, found 1198.4788.

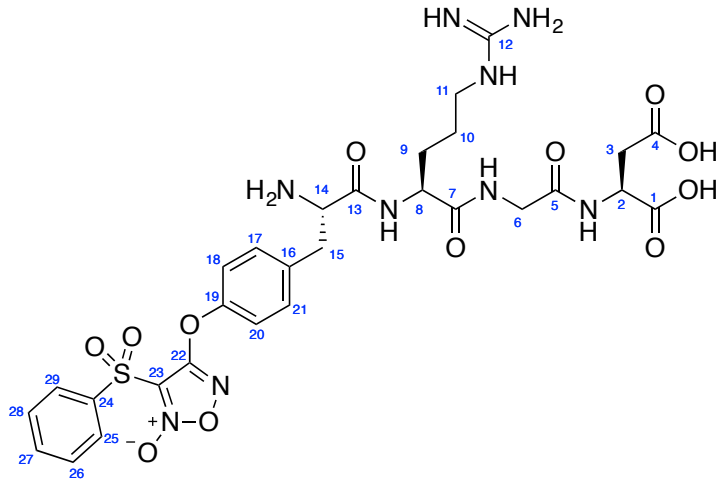
Tyr(*O*-carbonyl-*N*-3-phenylsydnonimine)-Arg-Gly-Asp trifluoroacetate, 348



Following general procedure C with Boc-Tyr(*O*-carbonyl-*N*-3-phenylsydnonimine)-Arg(Pbf)-Gly-Asp(OtBu)-OtBu **346** (60 mg, 52 µmol) and deprotecting cocktail (700 µL), to provide *Tyr(O-carbonyl-N-3-phenylsydnonimine)-Arg-Gly-Asp trifluoroacetate* **348** (38 mg, 48 µmol, 92%) as a white powder: R_f 0.00 (90:10, CH_2Cl_2:MeOH, UV/cerium phosphomolydate); **m.p.** 128-132 °C (decomp.); $[\alpha]_D^{20}$ +8.4, (*c* = 0.13, 1:1, H_2O:MeOH**;** ν_{max} (KBr disc)/cm^{-1} 2953, 2924, 2853, 1702, 1674, 1568, 1521, 1508, 1465, 1376, 1201, 1139, 1048; **^{1}H NMR** (500 MHz, d_6-DMSO) δ 8.80 (1H, d, *J* 7.7 Hz, N*H*-Tyr-Arg), 8.62 (1H, s, C*H*-24), 8.38 (1H, t, *J* 5.2 Hz, N*H*-Arg-Gly), 8.22 (1H, d, *J* 7.4 Hz, N*H*-Gly-Asp), 8.07 (2H, d, *J* 7.7 Hz, C*H*-26,30), 7.79 (1H, tt. *J* 7.5, 2.5 Hz, C*H*-28), 7.73 (2H, t, *J* 8.0 Hz, C*H*-27,29), 7.35-7.01 (7H, m, C*H*-17,18,20,21, 3 x N*H*-guanidine), 4.46 (1H, dd, *J* 12.2, 5.9 Hz, C*H*-2), 4.38 (1H, dd, *J* 13.0, 6.6, C*H*-8), 4.08 (1H, t, *J* 7.7 Hz, C*H*-14), 3.87 (1H, dd, *J* 17.7, 5.7, CH_AH_B-6), 3.69 (1H, dd, *J* 16.2, 5.2 Hz, CH_AH_B-6), 3.15-3.07 (3H, m, CH_2-11, CH_AH_B-15), 2.93 (1H, dd, *J* 15.0, 7.9 Hz, CH_AH_B-15), 2.60 (2H, d, *J* 5.6 Hz, CH_2-3), 1.80-1.73 (1H, m, CH_AH_B-9) and 1.67-1.48 (3H, m, CH_AH_B-9,

CH_2-10); **^{13}C NMR** (125 MHz, d_6-DMSO) δ 175.0 (quat., C-23), 172.6, 172.2, 171.0, 168.4, 168.1 (quat., × 5, C-1,4,5,7,13), 158.7 (quat., C-22), 156.8 (quat., C-12), 151.2 (quat., C-19), 133.6 (quat., C-25), 133.1 (*C*H, C-28), 131.3 (quat., C-16), 130.4 (*C*H × 2, C-17,21), 130.3 (*C*H × 2, C-27, 29), 122.6 (*C*H × 2, C-26,30), 121.8 (*C*H × 2, C-18,20), 104.9 (*C*H, C-24); 53.4 (*C*H_2, C-14), 52.5 (*C*H, C-8), 48.7 (*C*H, C-2), 41.6 (*C*H_2, C-6), 40.5 (*C*H_2, C-11), 36.7 (*C*H_2, C-3), 36.4 (*C*H_2, C-15), 29.6 (*C*H_2, C-9) and 24.8 (*C*H_2, C-10); ***m/z*** (ES$^+$) 697 ([M+H]$^+$, 100%); **HRMS** *m/z* (ES$^+$) calcd. for $C_{30}H_{37}N_{10}O_{10}$ [M+H]$^+$ requires 697.2689, found 697.2684.

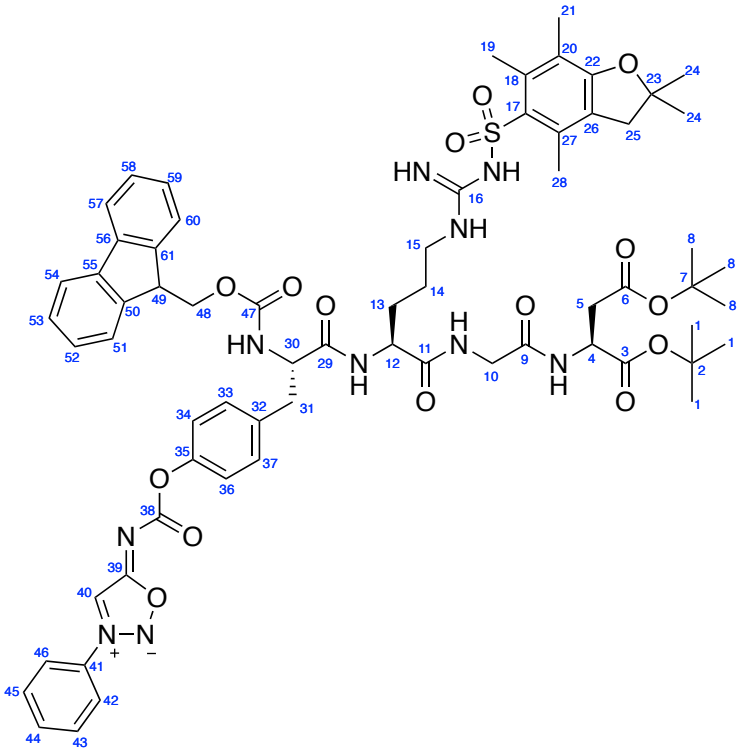
Tyr((*O*-(3-(phenylsulfonyl)-1,2,5-oxadiazole-2-oxide))-Arg-Gly-Asp trifluoroacetate, 349



Following general procedure C with Boc-Tyr((*O*-(3-(phenylsulfonyl)-1,2,5-oxadiazole-2-oxide))-Arg(Pbf)-Gly-Asp(OtBu)-OtBu **347** (60 mg, 50 μmol) and deprotecting cocktail (700 μL), to provide *Tyr((O-(3-(phenylsulfonyl)*-1,2*,5-oxadiazole-2-oxide))-Arg-Gly-Asp trifluoroacetate* **349** (37 mg, 45 μmol, 90%) as a white solid: ***R$_f$***. 0.00 (90:10, CH_2Cl_2:MeOH, UV/cerium phosphomolydate); **m.p.** 120-128 °C (decomp.); **[α]$_D^{20}$** +44.4, (*c* = 0.2, 1:1, H_2O:MeOH; **ν$_{max}$** (KBr)/cm^{-1} 3417, 2952, 2923, 2853, 1726, 1659, 1602, 1566, 1468, 1369, 1369, 1201, 1046; **^{1}H NMR** (500 MHz, d_6-DMSO) δ 8.80 (1H, d, *J* 7.9 Hz, N*H*-Tyr-Arg), 8.44 (1H, t, *J* 5.6 Hz, N*H*-Arg-Gly), 8.19 (1H, d, *J* 7.6 Hz, N*H*-Gly-Asp), 8.05 (2H, d, *J* 8.8 Hz, C*H*-25,29), 7.93 (1H, tt, *J* 7.4, 1.3 Hz, C*H*-27), 7.77 (2H, t, *J* 8.0 Hz, C*H*-28,26), 7.40-7.14 (7H, m, C*H*-17,18,20,21, 3 × N*H*-guanidine), 4.40 (1H, dd, *J* 13.3, 6,1 Hz, C*H*-2), 4.37 (1H, dd, *J* 13.2, 7.2, C*H*-8), 4.10 (1H, t, *J* 7.0 Hz, C*H*-14), 3.87 (1H, dd, *J* 16.6, 6.1 Hz, CH_AH_B-6), 3.77 (1H, dd, *J* 17.0, 5.4 Hz, CH_AH_B-6), 3.18-3.06 (3H m, CH_2-11, CH_AH_B-15), 2.97 (1H, dd, *J* 14.0, 8.0 Hz, CH_AH_B-15), 2.59 (2H, d, *J* 5.9, CH_2-3) 1.84-1.74 (1H, m, CH_AH_B-9) and 1.66-1.48 (3H, m, CH_AH_B-9, CH_2-10); **^{13}C NMR** (100 MHz, d_6-DMSO) δ 172.6, 172.3, 171.1, 168.3, 168.0 (quat., × 5, C-1,4,5,7,13), 158.4 (quat., C-22), 156.9 (quat., C-12), 151.9 (quat., C-19), 136.7 (quat., C-

24), 133.4 (quat., C-16), 131.3 (*C*H × 2, C-17,21), 130.1 (*C*H × 2, C-26,30), 128.6 (*C*H × 2, C-25,29), 119.7 (*C*H × 2, C-18,20), 111.3 (quat., C-23), 53.3 (*C*H, C-14), 52.5 (*C*H, C-8), 48.7 (*C*H, C-2), 41.7 (CH_2, C-6), 40.5 (CH_2, C-11), 36.9 (CH_2, C-3), 36.3 (CH_2, C-15), 29.6 (CH_2, C-9), 24.8 (CH_2, C-10); ***m/z*** (ES^+) 734 ($[M+H]^+$, 100%); **HRMS** *m/z* (ES^+) calcd. for $C_{29}H_{36}N_9O_{12}S$ $[M+H]^+$ requires 734.2199, found 734.2194.

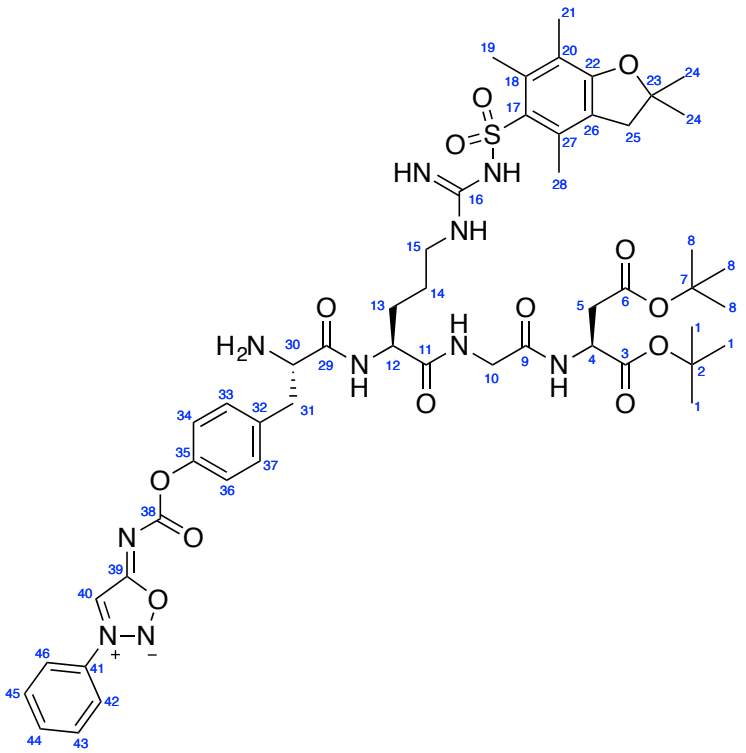
Fmoc-Tyr(*O*-carbonyl-*N*-3-phenylsydnonimine)-Arg(Pbf)-Gly-Asp(OtBu)-OtBu, 350



Following general procedure B, with HATU (749 mg, 1.97 mmol), NH_2-Arg(Pbf)-Gly-Asp(OtBu)-OtBu **341** (700 mg, 0.99 mmol), (*S*)-*N*-(((4-(2-(((9*H*-fluoren-9-yl)methoxy)carbonyl)amino)-2-carboxyethyl)phenoxy)carbonyl)-3-phenylsydnonimine **321** (581 mg, 0.99 mmol) and diisopropylethylamine (distilled, 381 mg, 510 µL, 2.95 mmol). Purification by silica gel chromatography, eluting with dichloromethane and methanol (100:0 to 97:3) provided *Fmoc-Tyr(O-carbonyl-N-3-phenylsydnonimine)-Arg(Pbf)-Gly-Asp(OtBu)-OtBu* **350** (965 mg, 0.75 mmol, 76%) as a white solid: $\boldsymbol{R_f}$ 0.71 (90:10, CH_2Cl_2:MeOH, UV/cerium phosphomolydate); **m.p.** 110-112 °C; $[\boldsymbol{\alpha}]_{\mathbf{D}}^{\mathbf{20}}$ +7.6, (*c* = 1.0, $CHCl_3$); $\boldsymbol{\nu_{max}}$ (thin film)/cm^{-1} 2982, 2934, 1723, 1699, 1685, 1668, 1603, 1587, 1539, 1509, 1369, 1265, 1217, 1191, 1106, 972; **^{1}H NMR** (500 MHz, $CDCl_3$) δ 8.24 (1H, s, C*H*-40), 7.83 (2H, d, *J* 8.1 Hz, C*H*-54,57), 7.70 (2H, d, *J* 7.6 Hz, C*H*-42,46), 7.66 (1H, t, *J* 7.6 Hz, C*H*-44), 7.60 (2H, t, *J* 7.6 Hz, C*H*-43,45), 7.53 (2H,

d, *J* 7.4 Hz, C*H*-51,60), 7.35-7.31 (3H, m, C*H*-53,58, N*H*-Gly-Asp-amide), 7.26-7.22 (3H, m, C*H*-52,59, N*H*-Tyr-Arg), 7.19 (2H, d, *J* 8.0 Hz, C*H*-33,37), 7.07 (2H, d, *J* 8.0 Hz, C*H*-34, 36), 6.36 (2H, s, *br*, N*H*-guanidine × 2), 6.01 (1H, s, *br*, N*H*-Fmoc), 4.66-4.62 (1H, m, C*H*-4), 4.59-4.54 (1H, m, C*H*-30), 4.50-4.42 (1H, m, C*H*-12), 4.36 (1H, t, *J* 8.6 Hz, CH_AH_B-48), 4.20 (1H, t, *J* 8.6 Hz, CH_AH_B-48), 4.09 (1H, t, *J* 8.6 Hz, C*H*-49), 3.96-3.90 (2H, m, CH_2-10), 3.18-3.06 (4H, m, CH_2-15, CH_2-31), 2.88 (2H, s, CH_2-25), 2.78 (1H, dd, *J* 17.6, 5.8 Hz, CH_AH_B-5), 2.67 (1H, dd, *J* 17.6, 4.7 Hz, CH_AH_B-5), 2.53 (3H, s, CH_3-19), 2.46 (3H, s, CH_3-21), 2.02 (3H, s, CH_3-28), 1.90-1.82 (1H, m, CH_AH_B-13), 1.70-1.62 (1H, m, CH_AH_B-13) and 1.45-1.35 (26H, m, 3 × CH_3-1, 3 × CH_3-8 Hz, CH_2-14, 2 × CH_3-24); **^{13}C NMR** (125 MHz, $CDCl_3$) δ 175.3 (quat., C-39), 172.1, 171.6, 169.8, 169.6, 169.0 (quat., × 5, C-3,6,9,11,29), 159.7 (quat., C-38), 158.4 (quat., C-22), 156.2 (quat., C-16), 156.2 (quat., C-47), 150.6 (quat., C-35), 143.6 (quat., × 2, C-55,56), 140.9 (quat., × 2, C-50,61), 138.0 (quat., C-17), 133.4 (quat., C-41), 133.4 (quat., C-32), 132.9 (*C*H, C-44), 132.8 (quat., C-26), 132.0 (quat., C-20), 130.3 (*C*H × 2, C-42,46), 130.1 (*C*H × 2, C-33,37), 127.5 (*C*H × 2, C-53,58), 126.9 (*C*H × 2, C-52,59), 125.1 (*C*H × 2, C-51,60), 124.4 (quat., C-18), 121.7 (*C*H × 2, C-34, 36), 121.5 (*C*H × 2, C-43,45), 119.7 (*C*H × 2, C-54,57), 117.2 (quat., C-27), 103.6 (*C*H, C-40), 86.1 (quat., C-23), 82.1 (quat., C-7), 81.2 (quat., C-2), 67.1 (*C*H_2, C-48), 56.1 (*C*H, C-30), 53.0 (*C*H, C-12), 49.3 (*C*H, C-4), 46.7 (*C*H, C-49), 43.0 (*C*H_2, C-25), 42.6 (*C*H_2, C-10), 40.3 (*C*H_2, C-15), 37.8 (*C*H_2, C-31), 37.3 (*C*H_2, C-5), 29.2 (*C*H_2, C-13), 28.4 (*C*H_3 × 2, C-24), 27.8 (*C*H_3 × 3, C-8), 27.7 (*C*H_3 × 3, C-1), 25.1 (*C*H_2, C-14), 19.2 (*C*H_3, C-21, 17.8 (*C*H_3, C-19) and 12.3 (*C*H_3, C-28); ***m/z*** (ES^+) 1283 ($[M+H]^+$, 100%); **HRMS** *m/z* (ES^+) calcd. for $C_{66}H_{78}N_{10}O_{15}S$ $[M+H]^+$ requires 1283.5442, found 1283.5423.

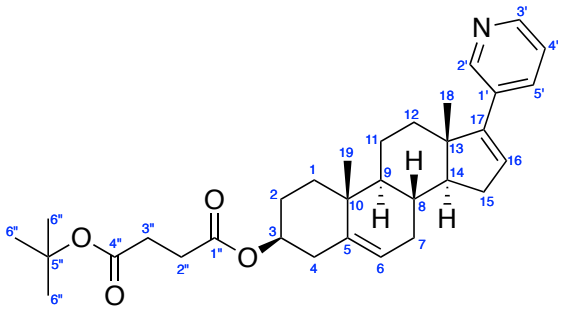
NH_2-Tyr(*O*-carbonyl-*N*-3-phenylsydnonimine)-Arg(Pbf)-Gly-Asp(OtBu)-OtBu, 351



Piperidine (350 µL, 3.51 mmol) was added to a solution of Fmoc-tetrapeptide **350** (300 mg, 0.23 mmol) in dry DMF (10 mL) cooled in an ice-bath. After addition, the ice-bath was removed and the solution stirred for 15 min. The solvent was evaporated under reduced pressure to yield a yellow residue. The residue suspended in dichloromethane and adsorbed onto silica gel. Purification by silica gel chromatography eluting with dichloromethane and methanol (100:0 to 90:0) provided amine **351** (224 mg, 0.211, 90%) as a pale yellow solid: $\boldsymbol{R_f}$ 0.44 (90:10, CH_2Cl_2:MeOH, UV/cerium phosphomolydate); **m.p.** 116-120 °C; $[\boldsymbol{\alpha}]_{\boldsymbol{D}}^{\boldsymbol{20}}$ -0.7, (*c* = 1.0, $CHCl_3$); $\boldsymbol{\nu_{max}}$ (thin film)/cm^{-1} 3334, 2976, 2932, 1728, 1698, 1654, 1583, 1554, 1509, 1419, 1367, 1279, 1214, 1190, 1155, 972; **^{1}H NMR** (500 MHz, $CDCl_3$) δ 8.31 (1H, s, C*H*-40), 7.87 (2H, d, *J* 7.7 Hz, C*H*-42,46), 7.72-7.61 (4H, m, C*H*-43,44,45, N*H*-Tyr-Arg), 7.20-7.11 (3H, m, C*H*-33,37, N*H*-Gly-Asp), 7.07 (2H, d, *J* 8.0 Hz, C*H*-35,36), 6.48 (2H, s, *br*, N*H*-guanidine), 6.12 (1H, s, *br*, N*H*-sulfonamide), 4.62 (1H, dt, *J* 8.5, 4.5 Hz, C*H*-4), 4.44-4.36 (1H, m, C*H*-12), 3.96 (1H, dd, *J* 5.6 Hz, *J* 17.2 Hz, CH_AH_B-10), 3.86 (1H, dd, *J* 16.8, 5.6 Hz, CH_AH_B-10), 3.77-3.74 (1H, m, C*H*-30), 3.19-3.13 (2H, m, CH_2-15), 3.05-2.99 (1H, m, CH_AH_B-31), 2.95-2.88 (3H, m, CH_2-25, CH_AH_B-31), 2.80 (1H, dd, *J* 17.2, 4.3, CH_AH_B-5), 2.68 (1H, dd, *J* 17.2, 4.3 Hz, CH_AH_B-5), 2.55 (3H, s, CH_3-19), 2.48 (3H, s, CH_3-21), 2.06 (3H, s, CH_3-28), 1.88-1.81 (1H, m, CH_AH_B-13), 1.66-1.58 (1H, m, CH_AH_B-13), 1.45-1.31 (26H, m, 3 × CH_3-1, 3 × CH_3-8 Hz, CH_2-14, 2 × CH_3-24); **^{13}C NMR** (125 MHz, $CDCl_3$) δ 175.7 (quat., C-39), 160.2 (quat., C-38), 158.6

(quat., C-22), 156.4 (quat., C-16), 150.7 (quat., C-35), 138.3 (quat., C-17), 134.0 (quat., C-32), 133.7 (quat., C-41), 133.3 (*C*H, C-44), 133.1 (quat., C-26), 132.3 (quat., C-20), 130.6 (*C*H × 2, C-43,45), 130.4 (*C*H × 2, C-33,37), 124.6 (quat., C-18), 122.0 (*C*H × 2, C-34,36), 121.6 (*C*H × 2, C-42,46), 117.4 (quat., C-27), 103.8 (*C*H, C-40), 86.3 (quat., C-23), 82.5 (quat., C-2), 81.6 (quat., C-7), 56.0 (*C*H, 30), 52.6 (*C*H, C-12), 49.2 (*C*H, C-4), 43.2 (CH_2, C-25), 42.8 (CH_2, C-10), 40.5 (CH_2, C-15), 37.4 (CH_2, C-5), 37.4 (CH_2, C-31), 29.1 (CH_2, C-13), 28.6 (CH_3 × 2, C-24), 28.0 (CH_3 × 3, C-8), 27.9 (CH_3 × 3, C-1), 25.2 (CH_2, C-14), 19.3 (CH_3, C-21), 18.0 (CH_3, C-19) and 12.5 (CH_3, C-28); ***m/z*** (ES^+) 1061 ($[M+H]^+$, 100%); **HRMS** *m/z* (ES^+) calcd. for $C_{51}H_{68}N_{10}O_{13}S$ $[M+H]^+$ requires 1061.4761, found 1061.4756.

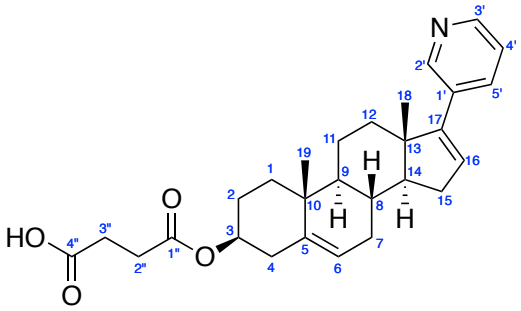
tert-Butyl-17-(3-Pyridyl)androsta-5,16-dien-3*β*-ol 3-hemisuccinate, 353



tert-Butyl hemisuccinate **264** (279 mg, 1.60 mmol) and DMAP (170 mg, 1.70 mmol) were added to a solution of abiraterone **106** (500 mg, 1.43 mmol) in dichloromethane (20 mL). EDCI.HCl (400 mg, 2.10 mmol) was added in one portion and the solution stirred for 6 h. The solution was washed with water and dried over $MgSO_4$. The mixture was filtered and the solvent adsorbed onto silica gel. Purification by silica gel chromatography, eluting with diethyl ether, provided *tert-butyl-17-(3- pyridyl)androsta*-5,16-*dien-3β-ol 3-hemisuccinate* **353** (686 mg, 1.36 mmol, 95%) as an white solid: R_f 0.66 (50:50, EtOAc:Cyclohexane, UV/cerium phosphomolydate); **m.p.** 209-211 °C; $[\alpha]_D^{20}$ -14.8, (*c* = 1.0, $CHCl_3$); ν_{max} (thin film)/cm^{-1} 1740, 1705, 1619, 1540, 1541, 1320, 1170, 1015; **^{1}H NMR** (500 MHz, $CDCl_3$) δ 8.56 (1H, d, *J* 1.5 Hz, C*H*-2′), 8.40 (1H, dd, *J* 4.7, 1.3 Hz, C*H*-6′), 7.59 (1H, d, *J* 7.9, 1.7 Hz, C*H*-4′), 7.16 (1H, dd, *J* 7.9, 4.7 Hz, C*H*-5′), 5.93 (1H, dd, *J* 3.0, 1.8 Hz, C*H*-16), 5.35 (1H, d, *J* 4.9 Hz, C*H*-6), 4.61-4.55 (1H, m, C*H*-3), 2.52-2.46 (4H, m, CH_2-2″,3″), 2.33-2.24 (2H, m, CH_2-1), 2.20 (1H, ddd, *J* 15.8, 6.6, 3.3 Hz, CH_AH_B-12), 2.03-1.96 (3H, m, CH_AH_B-7, CH_AH_B-12, CH_AH_B-15), 1.85-1.78 (2H, m, CH_AH_B-2, CH_AH_B-4), 1.74-1.36 (16H, m, CH_AH_B-2, 3 × CH_3-6″, CH_AH_B-7, C*H*-8,9, CH_2-11, CH_AH_B-15) and 1.13-0.97 (8H, m, CH_AH_B-4, C*H*-14, CH_3-18, CH_3-19); **^{13}C NMR** (125 MHz, $CDCl_3$) δ 172.9 (quat., C-1″), 172.1 (quat., C-4″), 151.6 (quat., C-17), 147.2 (*C*H × 2, C-2′,6′), 139.9 (quat., C-5), 133.6 (*C*H, C-4′), 132.9 (quat., C-1′), 129.2 (*C*H, C-16), 123.0

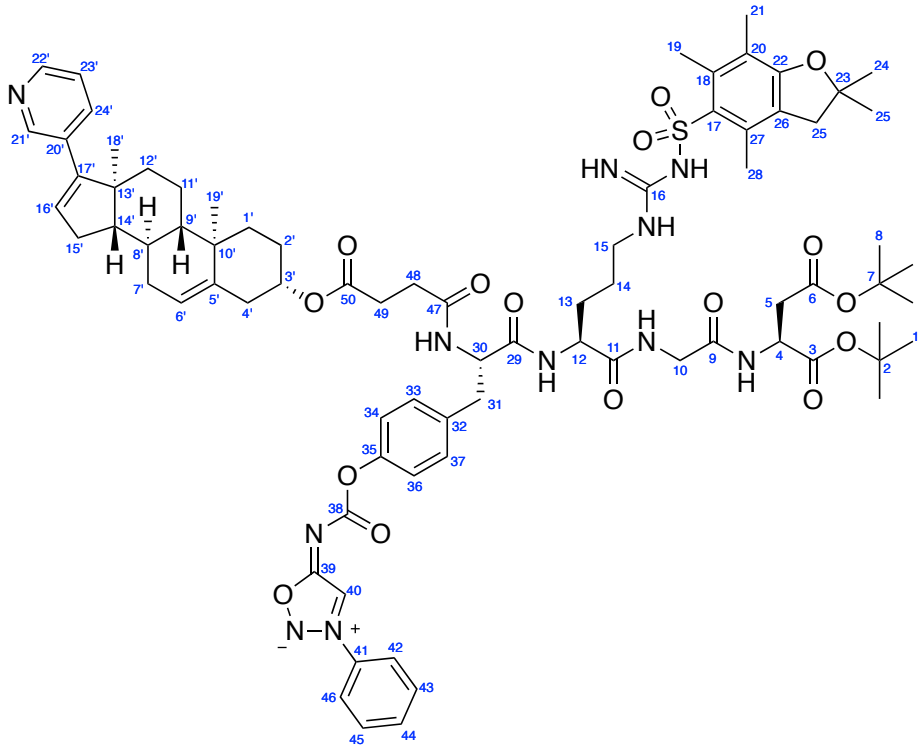
(*C*H, C-5′), 122.3 (*C*H, C-6), 80.6 (quat., C-5″), 74.0 (*C*H, C-3), 57.4 (*C*H, C-9), 50.2 (*C*H, C-14), 47.2 (quat., C-13), 38.1 (CH_2, C-1), 36.8 (CH_2, C-4), 36.7 (quat., C-10), 35.1 (CH_2, C-7), 31.8 (CH_2, C-12), 31.5 (CH_2, C-15), 30.3 (*C*H, C-8), 29.6 (CH_2 × 2, C-2″,3″), 28.0 (CH_3 × 3, C-6″), 27.7 (CH_2, C-2), 20.8 (CH_2, C-11), 19.2 (CH_3, C-19) and 16.5 (CH_3, C-18); ***m/z*** (ES^+) 1061 ($[M+H]^+$, 100%); **HRMS** *m/z* (ES^+) calcd. for $C_{32}H_{43}NNaO_4$ $[M+Na]^+$ requires 528.3084, found 528.3090.

17-(3-Pyridyl)androsta-5,16-dien-3*β*-ol 3-hemisuccinate, 352



Trifluoroacetic acid (2 mL) was added to a solution of *tert*-butyl-17-(3-pyridyl)androsta-5,16-dien-3*β*-ol 3-hemisuccinate **353** (200 mg, 0.40 mmol) in dichloromethane (10 mL) and stirred at room temperature overnight. The solvent was removed under reduced pressure to provide *17-(3-pyridyl)androsta*-5,16-*dien-3β-ol 3-hemisuccinate* **352** (164 mg, 0.50 mmol, 96%) as a white solid: R_f 0.00 (50:50, EtOAc:Cyclohexane, UV/$KMnO_4$); **m.p.** 238-240 °C; $[\alpha]_D^{20}$ +5.6, (*c* = 1.0, $CHCl_3$); ν_{max} (thin film)/cm^{-1} 3430, 1735, 1705, 1616, 1553, 1450, 1314, 1169, 1022; **^{1}H NMR** (500 MHz, $CDCl_3$) δ 8.64 (1H, s, *br*, C*H*-2′), 8.46 (1H, s, *br*, C*H*-6′), 7.71 (1H, dd, *J* 7.9, 1.8 Hz, C*H*-4′), 7.28 (1H, dd, *J* 7.9, 5.0 Hz, C*H*-5′), 6.02 (1H, dd, *J* 2.9, 1.6 Hz, C*H*-16), 5.40 (1H, d, *J* 4.6 Hz, C*H*-6), 4.68-4.61 (1H, m, C*H*-3), 2.70-2.60 (4H, m, CH_2-2″,3″), 2.36-2.33 (2H, m, CH_2-1), 2.20 (1H, ddd, *J* 16.1, 6.4, 3.2 Hz, CH_AH_B-12), 2.09-2.01 (3H, m, CH_AH_B-7, CH_AH_B-12, CH_AH_B-15), 1.89-1.83 (2H, m, CH_AH_B-2, CH_AH_B-4), 1.74 (1H, ddd, *J* 21.8, 10.7, 4.9 Hz, C*H*-8), 1.70-1.47 (6H, m, CH_AH_B-2, CH_AH_B-7, C*H*-9, CH_2-11, CH_AH_B-15) and 1.18-1.04 (8H, m, CH_AH_B-4, C*H*-14, CH_3-18, CH_3-19); **^{13}C NMR** (125 MHz, $CDCl_3$) δ 175.9 (quat., C-4″), 171.9 (quat., C-4″), 151.2 (quat., C-17), 146.5 (*C*H × 2, C-2′,6′), 140.0 (quat., C-5), 134.8 (*C*H, C-4′), 133.5 (quat., C-1′), 129.9 (*C*H, C-16), 123.5 (*C*H, C-5′), 122.3 (*C*H, C-6), 74.2 (*C*H, C-3), 57.4 (*C*H, C-9), 50.2 (*C*H, C-14), 47.3 (quat., C-13), 38.1 (CH_2, C-1), 36.9 (CH_2, C-4), 36.8 (quat., C-10), 35.1 (CH_2, C-7), 31.8 (CH_2, C-12), 31.5 (CH_2, C-15), 30.4 (*C*H, C-8), 29.6, 29.3 (CH_2 × 2, C-2″,3″), 27.7 (CH_2, C-2), 20.8 (CH_2, C-11), 19.3 (CH_3, C-19) and 16.6 (CH_3, C-18); ***m/z*** (ES^+) 1061 ($[M+H]^+$, 100%); **HRMS** *m/z* (ES^+) calcd. for $C_{28}H_{35}NNaO_4$ $[M+Na]^+$ requires 472.2458, found 472.2466.

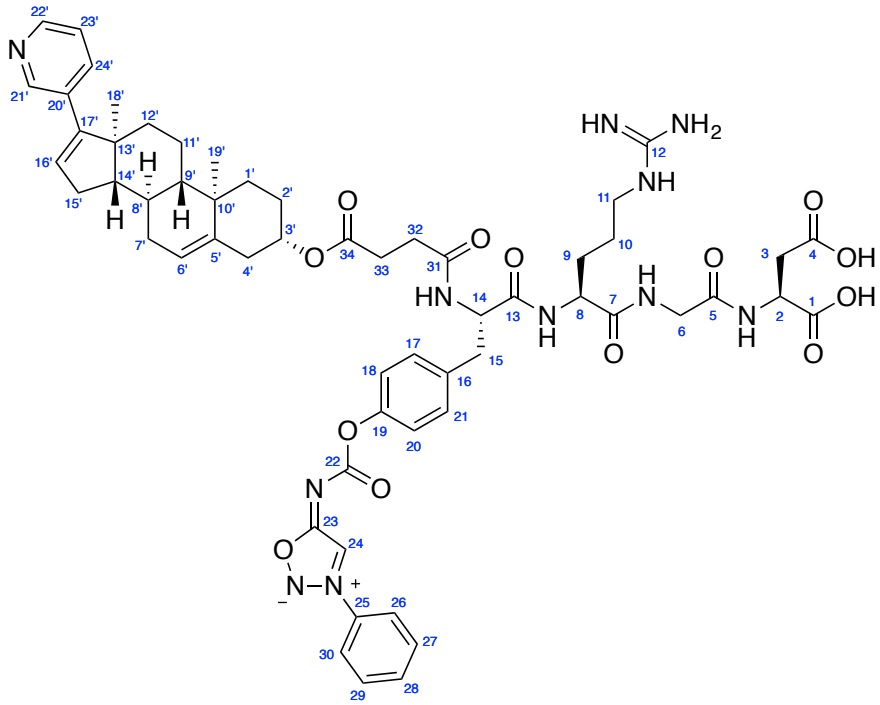
Pbf-(OtBu)$_2$-RGD-abiraterone hemsuccinamide, 354



Following general procedure B, with HATU (103 mg, 0.272 mmol), amine **351** (120 mg, 0.113 mmol), abiraterone hemisuccinate (74 mg, 0.135 mmol) and diisopropylethylamine (distilled, 59 mg, 75 μL, 0.453 mmol). Purification by silica gel chromatography, eluting with dichloromethane and methanol (100:0 to 97:3) provided hemisuccinamide **354** (167 mg, 0.11 mmol, 83%) as pale yellow solid: R_f 0.78 (90:10, CH_2Cl_2, UV/cerium phosphomolybdate), **m.p.** 45-49 °C, $[\alpha]_D^{20}$ -43.2, (*c* = 0.1, $CHCl_3$, ν_{max} (thin film)/cm^{-1} 1931, 2975, 1843, 1731, 1684, 1654, 1637, 1585, 1559, 1538, 1471, 1455, 1409, 1368, 1279, 1191, 1167; **^{1}H NMR** (500 MHz, CDCl3) δ 8.60 (1H, s, C*H*-21′), 8.45 (1H, d, *J* 4.8 Hz, C*H*-24′), 8.31 (1H, s, C*H*-40), 7.87 (2H, d, *J* 7.9 Hz, C*H*-42,46), 7.70-7.61 (4H, m, C*H*-43, 44,45,22′), 7.42 (1H, s, *br*, N*H*-amide), 7.32-7.21 (6H, m, C*H*-33,34,36,37,23′, N*H*-amide), 6.48 (2H, s, *br*, N*H*-guanidine), 6.00-5.98 (1H, m, C*H*-16′), 5.33 (1H, d, *J* 4.6 Hz, C*H*-6′), 4.72 (1H, dt, *J* 13.1, 5.3 Hz, C*H*-4), 4.67-4.63 (1H, m, C*H*-30), 4.52 (1H, ddd, *J* 10.5, 5.4, 5.1 Hz, C*H*-3′), 4.26-4.18 (1H, m, C*H*-12), 3.93-3.87 (2H, m, CH_2-10), 3.22-3.14 (1H, m, CH_AH_B-31), 3.13-3.02 (3H, m CH_2-15, CH_AH_B-31), 2.92 (2H, s, CH_2-25), 2.80 (1H, dd, *J* 17.0, 5.8, CH_AH_B-5), 2.73 (1H, dd, *J* 17.0, 5.1 Hz, CH_AH_B-5), 2.69-2.65 (1H, m, CH_AH_B-49), 2.57-2.39 (9H, s, CH_3-19, CH_3-21, CH_2-48, CH_AH_B-49), 2.31-2.21 (3H, m, CH_2-1′, CH_AH_B-12′), 2.11-1.79 (5H, m, CH_3-28, CH_AH_B-7′, CH_AH_B-12′), 1.93-1.21 (38H, m, 3 × CH_3-1, 3 × CH_3-8 , CH_2-13, CH_2-14, 2 × CH_3-24, CH_2-2′, CH_AH_B-4′, CH_AH_B-7′, C*H*-8′, C*H*-9′ Hz, CH_2-11′, CH_2-15′) and 1.17-0.98 (8H, m, CH_AH_B-4′, C*H*-14′, CH_3-18′, CH_3-19′); **^{13}C NMR** (125 MHz, $CDCl_3$) δ 175.6 (quat., C-39), 172.9, 172.9, 171.9, 171.8, 170.1,

169.8, 169.0 (quat., × 7, C-3,6,9,11,29,47,50), 160.0 (quat., C-38), 158.7 (quat., C-22), 156.3 (quat., C-16), 151.4 (quat., C-17′), 150.7 (quat., C-35), 147.4 (*C*H, C-24′), 147.4 (*C*H, C-21′), 139.7 (quat., C-5′), 138.3 (quat., C-17), 134.1 (*C*H, C-22′), 133.7 (quat., C-32), 133.6 (quat., C-41), 133.3 (*C*H, C-44), 133.2 (quat., C-20′), 133.0 (quat., C-26), 132.2 (quat., C-20), 130.6 (*C*H × 2, C-43,45), 130.3 (*C*H × 2, C-34,36), 129.6 (*C*H, C-16′), 124.6 (quat., C-18), 123.2 (*C*H, C-23′), 122.5 (*C*H, C-6′), 121.8 (*C*H × 2, C-33, 37), 121.6 (*C*H × 2, C-42,46), 103.8 (*C*H, C-40), 86.4 (quat., C-23), 81.6 (quat., C-3), 81.4 (quat., C-7), 74.5 (*C*H, C-3′), 57.4 (*C*H, C-9′), 56.3 (*C*H, C-30), 53.8 (*C*H, C-12), 50.9 (*C*H, C-14′), 49.4 (*C*H, C-4), 47.3 (quat., C-13′), 43.2 (CH_2, C-25), 42.8 (CH_2, C-10), 40.7 (CH_2, C-15), 37.9 (CH_2, C-1′), 37.5 (CH_2, C-31), 37.5 (CH_2, C-5), 36.8 (CH_2, C-4′), 36.7 (quat., C-10′), 35.1 (CH_2, C-7′), 31.8 (CH_2, C-12′), 31.5 (CH_2, C-15′), 30.3 (*C*H, C-8′), 29.8 (CH_2, C-13), 29.6 (CH_2, C-48), 29.6 (CH_2, C-49), 28.6 (CH_3 × 2, C-24), 28.1 (CH_3 × 3, C-1), 27.9 (CH_3 × 3, C-8), 27.7 (CH_2, C-2′), 25.5 (CH_2, C-14), 20.8 (CH_2, C-11′), 19.3 (CH_3, C-21), 19.2 (CH_3, C-19′), 18.0 (CH_3, C-19), 16.6 (CH_3, C-18′),12.5 (CH_3, C-27); ***m/z*** (ES^+) 1493 ($[M+H]^+$, 100%); **HRMS** *m/z* (ES^+) calcd. for $C_{79}H_{102}N_{11}O_{16}S$ $[M+H]^+$ requires 1492.7227, found 1492.7232.

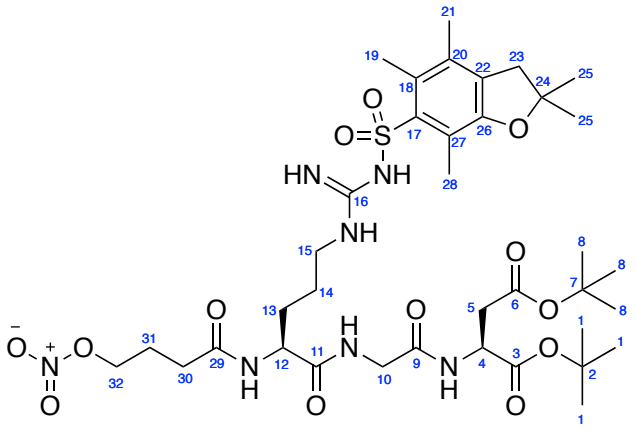
Abiraterone RGD-hemisuccinate, 355



Following general procedure C with Pbf-$(OtBu)_2$-RGD-abiraterone hemsuccinamide **354** (60 mg, 49 μmol) and deprotecting cocktail (700 μL), to provide *NO-abiraterone RGD-hemisuccinate* **355** (45 mg, 37 μmol, 75%) as a white powder: R_f 0.00 (90:10, CH_2Cl_2:MeOH,

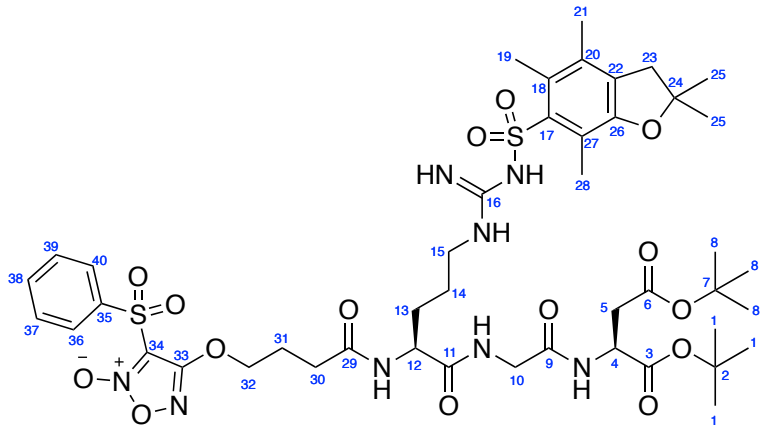
UV/cerium phosphomolydate); **m.p.** 159-163 °C; $[\alpha]_D^{20}$ -10.0, (*c* = 0.4, 1:1 H_2O:MeOH; ν_{max} (KBr disc)/cm^{-1} 2953, 2924, 2853, 1702, 1674, 1568, 1521, 1508, 1465, 1376, 1296, 1256, 1201; **^{1}H NMR** (500 MHz, d_6-DMSO) δ 8.78 (1H, s, *br*, C*H*-21′), 8.67-8.64 (2H, m, C*H*-24,24′), 8.28-8.21 (3H, m, C*H*-22′, N*H*-Tyr-Arg, N*H*-Gly-Asp), 8.15 (1H, t, *J* 5.8 Hz, N*H*-Arg-Gly), 8.08 (2H, d, *J* 8.5 Hz, C*H*-26,30), 7.79 (1H, tt, *J* 7.4, 2.5 Hz, C*H*-28), 7.76-7.72 (3H, m, C*H*-27,29,23′), 7.52 (1H, t, *J* 5.2 Hz, N*H*-succinamide), 7.39-6.75 (7H, m, C*H*-17, 18, 20, 21, 3 × N*H*-guanidine), 6.35 (1H, t, *J* 2.8 Hz, C*H*-16′), 5.38 (1H, d, *J* 4.0 Hz, C*H*-6′), 4.59-4.53 (2H, m, C*H*-2, 14′), 4.48-4.41 (1H, m, C*H*-3′), 4.31 (1H, dd, *J* 13.8, 7.4 Hz, C*H*-8), 3.83-3.74 (2H, m, CH_2-6), 3.12 (2H, dd, *J* 12.2, 6.3 Hz, CH_2-11), 3.04 (1H, dd, *J* 14.3, 3.5 Hz, C$H_A$$H_B$-15), 2.78 (1H, dd, *J* 13.9, 10.1 Hz, C$H_A$$H_B$-15), 2.70 (1H, dd, *J* 16.8, 5.9 Hz, C$H_A$$H_B$-3), 2.62 (1H, dd, *J* 16.8, 6.9 Hz, C$H_A$$H_B$-3), 2.44-2.33 (4H, m, C$H_2$-32,33), 2.29-2.22 (3H, m, CH_2-1′, C$H_A$$H_B$-12′), 2.14-1.96 (3H, m, C$H_A$$H_B$-7′, C$H_A$$H_B$-12′, C$H_A$$H_B$-15′), 1.86-1.31 (13H, m, CH_2-9,10, CH_2-1′, C$H_A$$H_B$-4′, C$H_A$$H_B$-7′, C*H*-8′,9′, C$H_2$-11′, C$H_A$$H_B$-15′) and 1.15-0.98 (8H, m, C$H_A$$H_B$-4′, C*H*-14′, C$H_3$-18′,19′); **^{13}C NMR** (500 MHz, d_6-DMSO) δ 174.9 (quat., C-23), 172.3, 171.7, 171.6, 171.4, 171.4, 170.9, 168.5 (quat., × 7, C-1,4,5,7,13,31,34), 159.4 (quat., C-17′), 158.7 (quat., C-22), 156.7 (quat., C-12), 150.5 (quat., C-19), 143.2 (*C*H, C-24′), 142.4 (*C*H, C-21′), 139.8 (quat., C-5′), 138.2 (*C*H, C-22′), 134.3 (quat., C-16), 133.8 (quat., C-20′), 133.6 (quat., C-25), 133.1 (*C*H, C-28), 132.0 (*C*H, C-16′), 130.3 (*C*H × 2, C-27,29), 129.8 (*C*H × 2, C-18,20), 125.4 (*C*H, C-23′), 122.4 (*C*H × 2, C-26,30), 121.8 (*C*H, C-6′), 121.3 (*C*H × 2, C-17,21), 104.7 (*C*H, C-24), 73.3 (*C*H, C-3′), 56.9 (*C*H, C-9′), 54.9 (*C*H, C-13), 52.3 (*C*H, C-8), 50.0 (*C*H, C-14′), 48.6 (*C*H, C-2), 46.7 (quat., C-13′), 41.6 (*C*H_2, C-6), 40.5 (*C*H_2, C-11), 37.7 (*C*H_2, C-1′), 36.7 (*C*H_2, C-15), 36.4 (*C*H_2, C-3), 36.3 (quat., C-10′), 36.1 (*C*H_2, C-4′), 34.2 (*C*H_2, C-7′), 31.5 (*C*H_2, C-12′), 30.9 (*C*H, C-15′), 29.9 (*C*H_2, C-9), 29.8 (*C*H, C-8′), 29.2, 29.1 (*C*H_2 × 2, C-32,33), 27.3 (*C*H_2, C-2′), 25.0 (*C*H_2, C-10), 20.3 (*C*H_2, C-11′), 18.9 (*C*H_3, C-19′) and 16.1 (*C*H_3, C-18′); **HRMS** *m/z* (ES^+) calcd. for $C_{58}H_{70}N_{11}O_{13}$ $[M+H]^+$ requires 1128.5149, found 1128.5148.

Pbf-(OtBu)$_2$-RGD-(4-nitrooxy)butyramide, 356



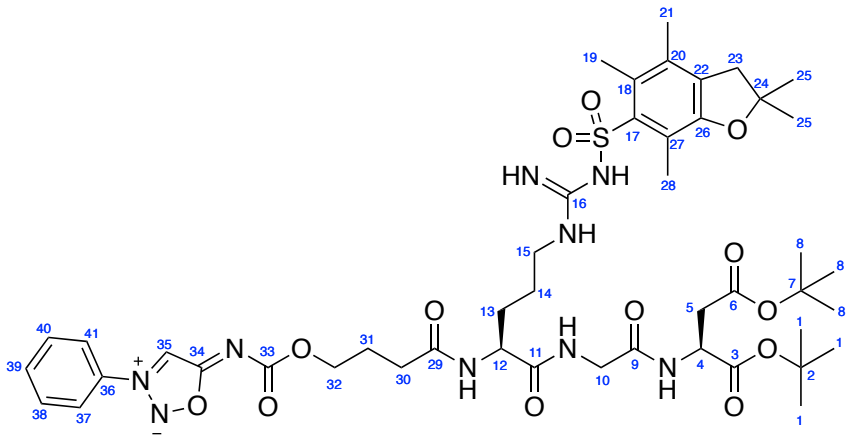
Following general procedure B, with HATU (161 mg, 0.423 mmol), NH_2-Arg(Pbf)-Gly-Asp(OtBu)-OtBu **341** (150 mg, 0.320 mmol), 4-nitrooxybutanoic acid **258** (31 mg, 0.211 mmol) and diisopropylethylamine (distilled, 82 mg, 110 µL, 0.633 mmol). Purification by silica gel chromatography, eluting with dichloromethane and methanol (100:0 to 97:3) provided *Pbf-(OtBu)$_2$-RGD-(4-nitrooxy)butyramide* **356** (115 mg, 0.136 mmol, 65%) as a colourless glass: $\boldsymbol{R_f}$ 0.61 (90:10, CH_2Cl_2:MeOH, UV/cerium phosphomolybdate); **m.p.** 78-81 °C; $[\boldsymbol{\alpha}]_{\boldsymbol{D}}^{\boldsymbol{20}}$ -6.2, (*c* = 0.1, $CHCl_3$); $\boldsymbol{\nu_{max}}$ (thin film)/cm^{-1} 1978, 2935, 1732, 1701, 1654, 1559, 1455, 1407, 1394, 1369, 1279, 1258, 1154, 1108; **^{1}H NMR** (500 MHz, $CDCl_3$) δ 7.76 (1H, t, *J* 5.0 Hz, N*H*-Arg-Gly), 7.33-7.28 (2H, m, N*H*-Gly-Asp, N*H*-amide), 6.43-6.14 (3H, m, 3 × N*H*-guanidine), 4.65 (1H, dt, *J* 8.3, 4.5 Hz, C*H*-4), 4.52-4.45 (3H, m, C*H*-12, CH_2-32), 3.97 (2H, d, *J* 5.1 Hz, CH_2-10), 3.30-3.17 (2H, m, CH_2-15), 2.94 (2H, s, CH_2-25), 2.80 (1H, dd, *J* 17.1, 5.3 Hz, CH_AH$_B$-5), 2.67 (1H, dd, *J* 17.1, 4.5 Hz, C$H_A$$H_B$-5), 2.55 (3H, s, C$H_3$-28), 2.48 (3H, s, C$H_3$-19), 2.39 (2H, t, *J* 7.2, CH_2-30), 2.07-2.01 (5H, m, CH_3-21,31), 1.93-1.85 (1H, m, CH_AH$_B$-13), 1.77-1.67 (1H, m, C$H_A$$H_B$-13), 1.64-1.53 (2H, m, CH_2-14), 1.46-1.38 (24H, m, 3 × CH_3-1, 3 × CH_3-8 Hz, 2 × CH_3-24); **^{13}C NMR** (125 MHz, $CDCl_3$) δ 172.8, 172.5, 170.2, 170.1, 169.2 (quat. × 5, C-3,6,9,11,29), 158.8 (quat., C-22), 156.6 (quat., C-16), 138.3 (quat., C-17), 132.7 (quat., C-26), 132.2 (quat., C-20), 124.7 (quat., C-18), 117.5 (quat., C-27), 86.4, 82.6, 81.6 (quat. × 3, C-2,7,23), 72.6 (CH_2, C-32), 53.1 (CH, C-12), 49.4 (CH, C-4), 43.2 (CH_2, C-25), 42.8 (CH_2, C-10), 40.4 (CH_3, C-15), 37.3 (CH_2, C-5), 31.8 (CH_2, C-30), 29.2 (CH_2, C-13), 28.6 (CH_3 × 2, C-24), 28.0, 27.9, (CH_3 × 6, C-1,8), 25.3 (CH_2, C-14), 22.6 (CH_2, C-31), 19.3 (CH_3, C-21), 18.0 (CH_3, C-19) and 12.5 (CH_3, C-28); ***m/z*** (ES$^+$) 842 ([M+H]$^+$, 100%); **HRMS** *m/z* (ES$^+$) calcd. for $C_{37}H_{60}N_7O_{13}S$ [M+H]$^+$ requires 842.3964, found 842.3966.

Pbf-(OtBu)$_2$-RGD-(4-(*O*-(3-(phenylsulfonyl)-1,2,5-oxadiazole 2-oxide)butyramide, 357



Following general procedure B, with HATU (149 mg, 0.391 mmol), NH$_2$-Arg(Pbf)-Gly-Asp(OtBu)-OtBu **341** (139 mg, 0.195 mmol), 4-(3-carboxypropoxy)-3-(phenylsulfonyl)-1,2,5-oxadiazole 2-oxide **261** (64 mg, 0.195 mmol) and diisopropylethylamine (distilled, 76 mg, 100 µL, 0.586 mmol). Purification by silica gel chromatography, eluting with dichloromethane and methanol (100:0 to 97:3) provided peptide **357** (151 mg, 0.148 mmol, 76%) as a white solid: $\boldsymbol{R_f}$ 0.64 (90:10, CH_2Cl_2:MeOH, UV/cerium phosphomolybdate); **m.p.** 69-71 °C, $[\alpha]_D^{20}$ +7.8, (*c* = 0.1, $CHCl_3$, $\boldsymbol{\nu_{max}}$ (thin film)/cm^{-1} 2979, 2935, 1734, 1652, 1557, 1450, 1409, 1369, 1256, 1169, 1167, 1089, 1107, 1023, 997; **^{1}H NMR** (500 MHz, $CDCl_3$) δ 8.00 (2H, d, *J* 7.5 Hz, C*H*-36,40), 7.80 (1H, s, *br*, N*H*-Arg-Gly), 7.69 (1H, t, *J* 7.7 Hz, C*H*-38), 7.56 (2H, t, *J* 7.8, C*H*-37,39), 7.35-7.28 (2H, m, N*H*-Gly-Asp, N*H*-amide), 6.39-6.17 (3H, m, 3 × N*H*-guanidine), 4.64-4.60 (1H, m, C*H*-4), 4.53-4.78 (1H, m, C*H*-12), 4.42-4.38 (2H, m, CH_2-32), 4.00-3.92 (2H, m, CH_2-10), 3.30-3.14 (2H, m, CH_2-15), 2.90 (2H, s, CH_2-25), 2.77 (1H, dd, *J* 17.0, 5.3, CH_AH$_B$-5), 2.66 (1H, dd, *J* 17.0, 3.7 Hz, C$H_A$$H_B$-5), 2.54-2.44 (8H, m, C$H_3$-19, C$H_3$-28,30), 2.17-2.11 (5H, m, CH_3-21,31), 1.93-1.85 (1H, m, CH_AH$_B$-13), 1.75-1.65 (1H, m, C$H_A$$H_B$-13), 1.61-1.51 (2H, m, CH_2-14) and 1.42-1.32 (24H, m, 3 × CH_3-1, 3 × CH_3-8, 2 × CH_3-24); **^{13}C NMR** (125 MHz, $CDCl_3$) δ 172.9, 172.6, 170.1, 169.8, 169.3 (quat. × 5, C-3,6,9,11,29), 158.9 (quat., C-34), 158.8 (quat., C-22), 156.6 (quat., C-16), 138.3 (quat., C-17), 137.8 (*C*H, C-35), 135.8 (quat, C-38), 132.7 (quat., C-26), 132.2 (quat., C-20), 129.7 (*C*H × 2, C-36,40), 128.5 (*C*H × 2, C-37,39), 124.7 (quat., C-18), 117.5 (quat., C-27), 110.5 (*C*H, C-33), 86.4, 82.6, 81.6 (quat. × 3, C-2,7,23), 70.7 (*C*H$_2$, C-32), 53.1 (*C*H, C-12), 49.4 (*C*H, C-4), 43.2 (*C*H$_2$, C-25), 42.8 (*C*H$_2$, C-10), 40.3 (*C*H$_3$, C-15), 37.3 (*C*H$_2$, C-5), 31.6 (*C*H$_2$, C-30), 29.2 (*C*H$_2$, C-13), 28.6 (*C*H$_3$ × 2, C-24), 28.0, 27.8, (*C*H$_3$ × 6, C-1,8), 25.3 (*C*H$_2$, C-14), 24.2 (*C*H$_2$, C-31), 19.3 (*C*H$_3$, C-21), 18.0 (*C*H$_3$, C-19), 12.5 (*C*H$_3$, C-28); ***m/z*** (ES$^+$) 1021 ([M+H]$^+$, 100%); **HRMS** *m/z* (ES$^+$) calcd. for $C_{45}H_{65}N_8O_{15}S_2$ [M+H]$^+$ requires 1021.4005, found 1021.4008.

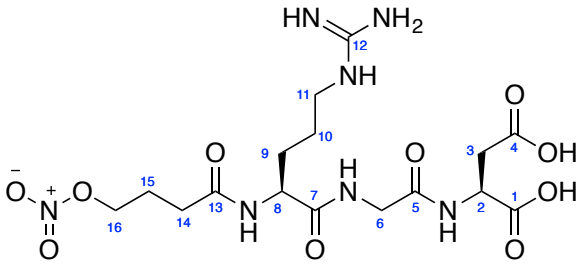
Pbf-(OtBu)$_2$-RGD-(4-(*O*-carbonyl-*N*-3-phenylsydnonimine)butyramide, 358



Following general procedure B, with HATU (145 mg, 0.38 mmol), NH_2-Arg(Pbf)-Gly-Asp(OtBu)-OtBu **341** (135 mg, 0.19 mmol), *N*-((3-carboxypropxy)carbonyl)-3-phenylsydnonimine **266** (55 mg, 0.19 mmol) and diisopropylethylamine (distilled, 76 mg, 100 µL, 0.59 mmol). Purification by silica gel chromatography, eluting with dichloromethane and methanol (100:0 to 97:3) provided *Pbf-(OtBu)$_2$-RGD-(4-(O-carbonyl-N-3-phenylsydnonimine)butyramide* **358** (150 mg, 0.16 mmol, 80%) as a pale yellow solid: $\boldsymbol{R_f}$ 0.64 (90:10, CH_2Cl_2:MeOH, UV/cerium phosphomolybdate); **m.p.** 109-114 °C; $[\alpha]_D^{20}$ -23.9, (*c* = 0.2, $CHCl_3$, $\boldsymbol{\nu_{max}}$ (thin film)/cm^{-1} 2934, 2978, 1734, 1700, 1684, 1654, 1651, 1635, 1575, 1561, 1456, 1394, 1369, 1267, 1201, 1153, 1106; **^{1}H NMR** (500 MHz, $CDCl_3$) δ 8.23 (1H, s, *br*, C*H*-35), 8.03 (1H, s, *br*, N*H*-amide), 7.84 (2H, d, *J* 7.4 Hz, C*H*-37,41), 7.64 (1H, t, *J* 7.5 Hz, C*H*-39), 7.59 (2H, t, *J* 7.8 Hz, C*H*-38,40), 7.40 (2H, s, *br*, N*H*-Arg-Gly, N*H*-Gly-Asp), 6.37-6.15 (3H, m, 3 × N*H*-guanidine), 4.67-4.60 (1H, m, C*H*-4), 4.44-4.36 (1H, m, C*H*-12), 4.19-4.05 (2H, m, CH_2-32), 4.03-3.96 (1H, m, CH_AH$_B$-10), 3.94-3.88 (1H, m, CH_A*H*$_B$-10), 3.24-3.16 (2H, m, CH_2-15), 2.90 (2H, s, CH_2-25), 2.74 (1H, dd, *J* 17.6, 6.0 Hz, CH_AH$_B$-5), 2.66 (1H, dd, *J* 17.6, 4.4 Hz, CH_A*H*$_B$-5), 2.51-2.49 (8H, m, CH_3-19, CH_3-28, CH_2-30), 2.04-1.98 (5H, m, CH_3-21, CH_2-31), 1.89-1.84 (1H, m, CH_AH$_B$-13), 1.77-1.66 (1H, m, CH_A*H*$_B$-13), 1.65-1.53 (2H, m, CH_2-14) and 1.42-1.32 (24H, m, 3 × CH_3-1, 3 × CH_3-8, 2 × CH_3-24); **^{13}C NMR** (125 MHz, $CDCl_3$) δ 175.0 (quat., C-34), 174.2, 173.4, 170.2, 170.1, 169.6 (quat. × 5, C-3,6,9,11,29), 161.2 (quat., C-33), 158.7 (quat., C-22), 156.5 (quat., C-16), 138.3 (quat., C-17), 133.7 (quat., C-26), 133.1 (*C*H, C-39), 132.7 (quat, C-26), 132.2 (quat., C-20), 130.4 (*C*H × 2, C-37,41), 124.7 (quat., C-18), 121.8 (*C*H × 2, C-38,40), 117.5 (quat., C-27), 103.7 (*C*H, C-35), 86.3, 86.2, 81.6 (quat. × 3, C-2,7,23), 65.0 (*C*H$_2$, C-32), 53.8 (*C*H, C-12), 49.4 (*C*H, C-4), 43.2 (*C*H$_2$, C-25), 42.8 (*C*H$_2$, C-10), 40.4 (*C*H$_3$, C-15), 37.7 (*C*H$_2$, C-5), 32.1 (*C*H$_2$, C-30), 28.7 (*C*H$_2$, C-13), 28.6 (*C*H$_3$ × 2, C-24), 28.0, 28.0, (*C*H$_3$ × 6, C-1,8), 25.5 (*C*H$_2$, C-14), 24.6 (*C*H$_2$, C-31), 19.3 (*C*H$_3$, C-21), 18.0

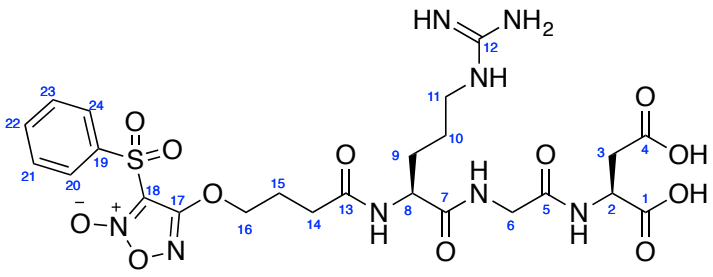
(CH_3, C-19) and 12.5 (CH_3, C-28); ***m/z*** (ES^+) 984 ($[M+H]^+$, 100%); **HRMS** *m/z* (ES^+) calcd. for $C_{46}H_{66}N_9O_{13}S$ $[M+H]^+$ requires 984.4495, found 984.4500.

RGD-(4-Nitrooxy)butyramide trifluoroacetate, 359



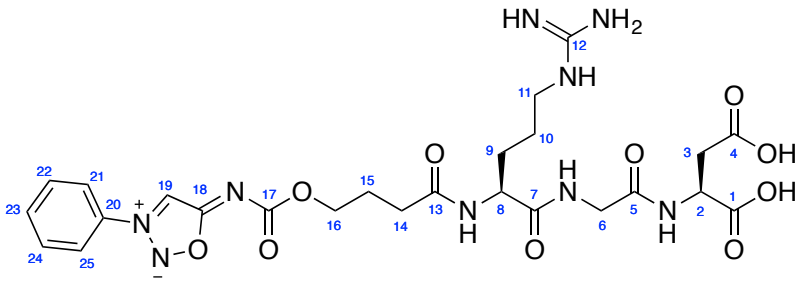
Following general procedure C with Pbf-$(OtBu)_2$-RGD-(4-nitrooxy)butyramide **356** (60 mg, 71 μmol) and deprotecting cocktail (700 μL), to provide *RGD-(4-nitrooxy)butyramide trifluoroacetate* **359** (35 mg, 61 μmol, 85%) as the white powder: $\boldsymbol{R_f}$ 0.00 (90:10, CH_2Cl_2, UV/cerium phosphomolybdate); **m.p.** 136 °C (decomp); $[\boldsymbol{\alpha}]_{\boldsymbol{D}}^{\boldsymbol{20}}$ +4.2, (*c* = 0.3, 1:1 H_2O:MeOH; $\boldsymbol{\nu_{max}}$ (KBr disc)/cm^{-1} 3209, 1700, 1653, 1559, 1506, 1457, 1110; **^{1}H NMR** (500 MHz, d_6-DMSO) δ 11.85 (2H, s, *br*, 2 × CO_2H), 8.25-8.13 (3H, m, 3 × N*H*-backbone), 7.54 (1H, s, *br*, N*H*-guanidine), 7.46-6.71 (4H, m, NH_2-guanidine, N*H*-guanidine, CF_3CO_2H), 4.55-4.78 (3H, m, C*H*-2, CH_2-16), 4.23 (1H, dd, *J* 13.4, 6.2 Hz, C*H*-8), 3.72 (2H, d, *J* 5.7 Hz, CH_2-6), 3.08 (1H, dd, *J* 13.0, 6.7 Hz, CH_2-11), 2.66 (1H, dd, *J* 16.7, 6.7 Hz, CH_AH_B-3), 2.57 (1H, dd, *J* 16.7, 6.4 Hz, CH_AH_B-3), 2.07 (2H, t, *J* 7.4 Hz, CH_2-14), 1.89 (1H, tt, *J* 7.0, 6.8 Hz, CH_2-15), 1.72-1.62 (1H, m, CH_AH_B-9) and 1.54-1.43 (3H, m, CH_AH_B-9, CH_2-10); **^{13}C NMR** (125 MHz, d_6-DMSO) 172.3, 171.8, 171.6, 171.5, 168.5 (quat. × 5, C-1,4,5,7,13), 156.6 (quat., C-12), 73.7 (CH_2, C-16), 53.4 (CH, C-8), 48.6 (CH, C-2), 41.6 (CH_2, C-6), 40.5 (CH_2, C-11), 36.1 (CH_2, C-3), 31.4 (CH_2, C-14), 29.0 (CH_2, C-9), 25.0 (CH_2, C-10) and 22.3 (CH_2, C-15); ***m/z*** (ES^+) 697 ($[M+H]^+$, 100%); **HRMS** *m/z* (ES^+) calcd. for $C_{18}H_{28}N_7O_{10}$ $[M+H]^+$ requires 479.1892, found 478.1880.

RGD-(4-(*O*-(3-(Phenylsulfonyl)-1,2,5-oxadiazole 2-oxide)butyramide trifluoroacetate, 360



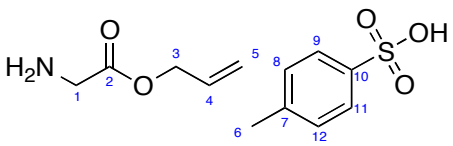
Following general procedure C with Pbf-(OtBu)$_2$-RGD-(4-(*O*-(3-(phenylsulfonyl)-1,2,5-oxadiazole 2-oxide)butyramide **357** (90 mg, 88 μmol) and deprotecting cocktail (900 μL), to provide *RGD-(4-(O-(3-(phenylsulfonyl)*-1,2,*5-oxadiazole 2-oxide)butyramide trifluoroacetate* **360** (60 mg, 79 μmol, 90%) as a white powder: R_f 0.00 (90:10, CH_2Cl_2, UV/cerium phosphomolybdate); **m.p.** 115-121 °C (decomp); $[\alpha]_D^{20}$+11.4, (*c* = 0.1, 1:1 H_2O:MeOH; ν_{max} (KBr disc)/cm^{-1} 2953, 2923, 2853, 1727, 1605, 1568, 1456, 1378, 1291, 1168, 1085; **^{1}H NMR** (500 MHz, d6-DMSO) δ 11.97 (2H, s, *br*, 2 × CO_2H), 8.29 (1H, t, *J* 5.9 Hz, N*H*-Arg-Gly), 8.20-8.16 (2H, m, N*H*-amide-Arg, N*H*-Gly-Asp), 8.04 (2H, d, *J* 7.6 Hz, C*H*-20,24), 7.92 (1H, tt, *J* 8.0, 1.4 Hz, C*H*-22), 7.77 (2H, t, *J* 7.8 Hz, C*H*-21,23), 7.69 (1H, s, *br*, N*H*-guanidine), 7.35-6.75 (4H, m, NH_2-guanidine, N*H*-guanidine, CF_3CO_2H), 4.52 (1H, dd, *J* 13.8, 6.1 Hz, C*H*-2), 4.42 (2H, t *J* 6.4 Hz, CH_2-16), 4.29 (1H, dd, *J* 13.4, 6.2 Hz, C*H*-2), 3.79 (1H, dd, *J* 17.0, 5.5, CH_AH$_B$-6), 3.72 (1H, dd, *J* 16.9, 5.6 Hz, C$H_A$$H_B$-6), 3.11 (2H, dd, *J* 14.0, 7.9 Hz, CH_2-11), 2.67 (1H, dd, *J* 16.8, 6.1 Hz, CH_AH$_B$-3), 2.59 (1H, dd, *J* 16.8, 6.4 Hz, C$H_A$$H_B$-3), 2.33 (2H, t, *J* 7.5 Hz, CH_2-14), 2.00 (1H, tt, *J* 6.9, 6.8 Hz, CH_2-15), 1.78-1.70 (1H, m, CH_AH$_B$-9) and 1.59-1.46 (3H, m, C$H_A$$H_B$-9, C$H_2$-10); **^{13}C NMR** (125 MHz, d_6-DMSO) 172.4, 171.9, 171.8, 171.5, 168.5 (quat. × 5, C-1,4,5,7,13), 158.9 (quat., C-17), 156.6 (quat., C-12), 137.1 (quat., C-19), 136.2 (*C*H, C-22), 130.1 (*C*H × 2, C-20,24), 128.4 (*C*H × 2, C-21,23), 110.6 (quat., C-18), 71.0 (*C*H_2, C-16), 52.3 (*C*H, C-8), 48.5 (*C*H, C-2), 41.6 (*C*H_2, C-6), 40.5 (*C*H_2, C-11), 36.4 (*C*H_2, C-3), 30.9 (*C*H_2, C-14), 29.2 (*C*H_2, C-9), 25.0 (*C*H_2, C-10) and 24.2 (*C*H_2, C-15); ***m/z*** (ES$^+$) 657 ([M+H]$^+$, 100%); **HRMS** *m/z* (ES$^+$) calcd. for $C_{24}H_{33}N_8O_{12}S$ [M+H]$^+$ requires 657.1933, found 657.1925.

RGD-(4-(*O*-Carbonyl-*N*-3-phenylsydnonimine)butyramide, 361



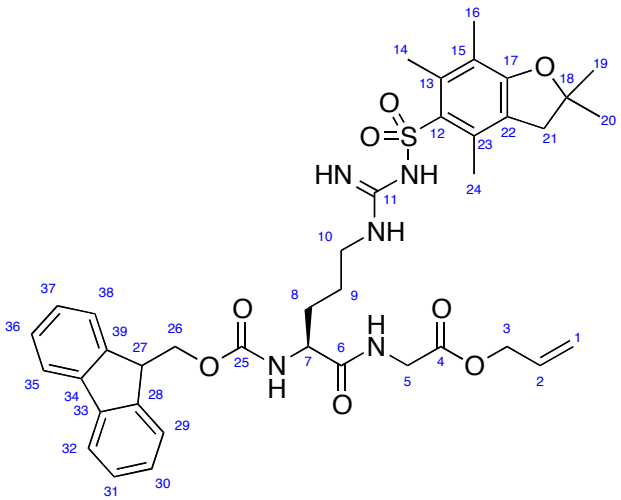
Following general procedure C with Pbf-(OtBu)$_2$-RGD-(4-(*O*-carbonyl-N-3-phenylsydnonimine)butyramide **358** (60 mg, 61 µmol) and deprotecting cocktail (600 µL), to provide *RGD-(4-(O-carbonyl-N-3-phenylsydnonimine)butyramide* **361** (38 mg, 54 µmol, 88%) as a white powder: $\boldsymbol{R_f}$ 0.00 (90:10, CH_2Cl_2, UV/cerium phosphomolybdate); **m.p.** 158-160 °C (decomp); $[\boldsymbol{\alpha}]_{\mathbf{D}}^{\mathbf{20}}$+8.0, (*c* = 0.2, 1:1 H_2O:MeOH; $\boldsymbol{\nu_{max}}$ (KBr disc)/cm^{-1} 2955, 2923, 2850, 1720, 1604, 1577, 1450, 1362, 1301, 1150; **^{1}H NMR** (500 MHz, d_6-DMSO) δ 8.63 (1H, s, C*H*-19), 8.22-8.12 (3H, m, 3 × N*H*-backbone), 8.07 (2H, d, *J* 7.7 Hz, C*H*-21,25), 7.78 (1H, tt, *J* 6.4, 1.4 Hz, C*H*-23), 7.73 (2H, t, *J* 8.0 Hz, C*H*-22,24), 7.48 (1H, t, *J*, N*H*-guanidine), 7.40-6.44 (4H, m, NH_2-guanidine, N*H*-guanidine, CF_3CO_2H), 4.55 (1H, dd, *J* 14.2, 6.0 Hz, C*H*-2), 4.24 (1H, dd, *J* 13.5, 6.3 Hz, C*H*-2), 4.03 (2H, t *J* 6.6 Hz, CH_2-16), 3.73 (2H, d, *J* 5.9 Hz, CH_2-6), 3.09 (2H, dd, *J* 13.2, 6.8 Hz, CH_2-11), 2.68 (1H, dd, *J* 13.9, 6.1 Hz, CH_AH$_B$-3), 2.58 (1H, dd, *J* 16.6, 6.7 Hz, C$H_A$$H_B$-3), 2.52 (2H, t, *J* 7.5 Hz, CH_2-14), 1.83 (1H, tt, *J* 7.2, 6.4 Hz, CH_2-15), 1.73-1.65 (1H, m, CH_AH$_B$-9) and 1.56-1.44 (3H, m, C$H_A$$H_B$-9, C$H_2$-10); **^{13}C NMR** (125 MHz, d_6-DMSO) 173.9 (quat., C-18), 172.3, 172.0, 171.8, 171.6, 168.6 (quat. × 5, C-1,4,5,7,13), 159.6 (quat., C-17), 156.6 (quat., C-12), 133.6 (quat., C-20), 133.1 (*C*H, C-23), 130.3 (*C*H × 2, C-22,24), 122.5 (*C*H × 2, C-21,25), 105.0 (*C*H, C-19), 64.3 (*C*H$_2$, C-16), 52.3 (*C*H, C-8), 48.5 (*C*H, C-2), 41.6 (*C*H$_2$, C-6), 40.4 (*C*H$_2$, C-11), 36.0 (*C*H$_2$, C-3), 31.3 (*C*H$_2$, C-14), 29.0 (*C*H$_2$, C-9), 25.0 (*C*H$_2$, C-10) and 24.7 (*C*H$_2$, C-15); ***m/z*** (ES$^+$) 620 ([M+H]$^+$, 100%); **HRMS** *m/z* (ES$^+$) calcd. for $C_{25}H_{34}N_9O_{10}$ [M+H]$^+$ requires 620.2423, found 620.2412.

NH_2-Gly-OAll *p*-toluenesulfonate, 367[420]



p-Toluenesulfonic acid (24.3 g, 135 mmol) was added to a solution of glycine **366** (9.4 g, 135 mmol) in toluene (50 mL) and allyl alcohol (65 mL). The mixture was heated to reflux using a Dean-Stark trap overnight. The resultant solution was cooled to room temperature and the solvent removed under reduced pressure. The residue was cooled in an ice-bath and diethyl ether added. The precipitant was collected to afford NH_2-Gly-OAll *p*-toluenesulfonate **367** (35.0 g, 122 mmol, 90%) as an off-white solid, which was used without any further purification: **m.p.** 130-132 °C; **^{1}H NMR** (500 MHz, $CDCl_3$) δ 8.06 (3H, s, *br*, N*H*$_2$.TsO*H*), 7.67 (2H, d, *J* 7.9 Hz, C*H*-8,12), 7.07 (2H, d, *J* 7.9 Hz, C*H*-9,11), 5.72 (1H, ddd, *J* 22.4, 10.5, 5.7 Hz, C*H*-4), 5.20 (1H, dd, *J* 16.0, 1.2 Hz, C*H*-5-*trans*), 5.14 (1H, dd, *J* 10.5, 1.0 Hz, C*H*-5-*cis*), 4.46 (2H, d, *J* 5.7 Hz, CH_2-3), 3.72 (2H, s, CH_2-1), 2.31 (3H, s, CH_2-6); **^{13}C NMR** (125 MHz, $CDCl_3$) δ 167.3 (quat., C-2), 141.1 (quat., C-7), 140.5 (quat., C-10), 131.1 (*C*H, C-4), 129.0 (*C*H × 2, C-8,12), 125.9 (*C*H × 2, C-9,11), 119.0 (*C*H_2, C-5), 66.6 (*C*H_2, C-3), 40.5 (*C*H_2, C-1) and 21.3 (*C*H_3, C-6); ***m/z*** 138 ([M+Na]$^+$, 100%). The data were in agreement with the literature values. [420]

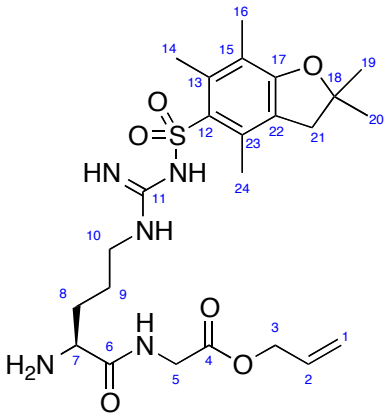
Fmoc-Arg(Pbf)-Gly-OAll, 368



HOBt hydrate (937 mg, 6.94 mmol) and triethylamine (702 mg, 1.0 mL, 6.94 mmol) were added to a solution of Fmoc-Arg(Pfb)-OH (1.5 g, 2.31 mmol) and NH_2-Gly-OAll *p*-toluenesulfonate **367** (995 mg, 3.47 mmol) in dry DMF (20 mL) cooled to 0 °C. After 15 min,

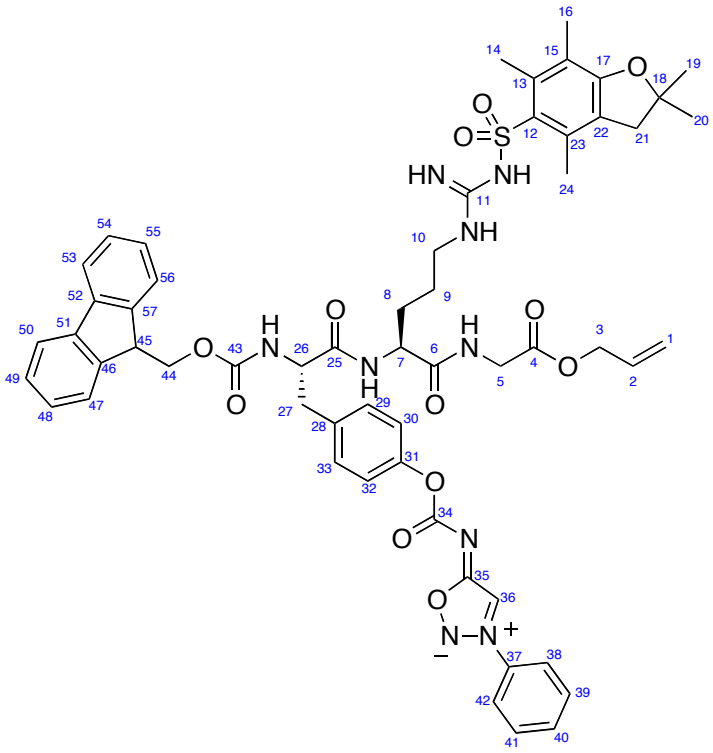
EDCI.HCl (1.33 g, 6.94 mmol) was stirred for a further 1 h at 0 °C. Following this time, the solution was allowed to warm to room temperature and stirred for 18 h. The resultant solution was poured into distilled water (200 mL) and extracted with ethyl acetate (4 × 30 mL). The organic layers were combined and washed with aq. HCl (2 N, 60 mL), saturated $NaHCO_3$ solution (60 mL), and brine (60 mL). The solution was dried over Na_2SO_4, filtered and the solvent removed under reduced pressure. The residue was uptaken in dichloromethane (50 mL) and adsorbed onto silica gel. Purification by silica gel chromatography eluting with dichloromethane and methanol (99:1 to 95:5) provided *Fmoc-Arg*(*Pfb)-Gly-OAll* **368** (1.54 g, 2.07 mmol, 89%) as white crystalline solid: $\boldsymbol{R_f}$ 0.65 (90:10, CH_2Cl_2:MeOH); **m.p.** 65-69 °C, $\boldsymbol{[\alpha]_D^{20}}$ -5.3 (*c* = 1.0, $CHCl_3$); $\boldsymbol{\nu_{max}}$ (thin film)/cm^{-1} 1730, 1690, 1550, 1451, 1410, 1380, 1245, 1110, 1034, 994; **^{1}H NMR** (500 MHz, $CDCl_3$) δ 7.73-7.69 (3H, m, C*H*-32,35, N*H*-Arg-Gly), 7.54 (2H, dd, *J* 7.2, 4.1 Hz, C*H*-29,36), 7.34 (2H, t, *J* 7.5 Hz, C*H*-31,36), 7.23 (2H, ddd, *J* 15.0, 7.5, *J* 2.9 Hz, C*H*-30, 37), 6.38 (2H, s, N*H*-guanidine, N*H*-sulfonamide), 6.19 (2H, , N*H*-Fmoc, N*H*-guanidine), 5.83 (1H, ddd, *J* 22.6, 11.1, 5.7 Hz, C*H*-2), 5.27 (1H, dd, *J* 16.5, 1.2 Hz, C*H*-1-*trans*), 5.19 (1H, dd, *J* 10.5, 1.0 Hz, C*H*-1-*cis*), 4.55 (2H, d, *J* 5.5 Hz, CH_2-3), 4.38 (1H, dd, *J* 8.1, 12.8 Hz, C*H*-7), 4.30 (2H, d, *J* 6.9 Hz, CH_2-26), 4.11 (1H, t, *J* 7.5 Hz, C*H*-27), 4.07 (1H, dd, *J* 18.0, 6.0 Hz, CH_AH_B-5), 3.92 (1H, dd, *J* 17.6, 5.5 Hz, CH_AH_B-5), 3.89-3.31 (1H, m, CH_AH_B-10), 3.24-3.17 (1H, m, CH_AH_B-10), 2.89 (2H, s, CH_2-21), 2.57 (3H, s, CH_2-24), 2.48 (3H, s, CH_2-14), 2.07 (3H, s, CH_2-16), 1.98-1.91 (1H, m, CH_AH_B-9), 1.77-1.70 (1H, m, CH_AH_B-9), 1.65-1.60 (2H, m, CH_2-8), 1.41 (6H, s, $CH_3 \times 2$, CH_2-19, 20); **^{13}C NMR** (125 MHz, $CDCl_3$) δ 173.0 (quat., C-4), 169.6 (quat., C-6), 158.8 (quat., C-22), 156.4 (quat., C-25), 156.3 (quat., C-11), 143.9, 143.7 (quat., × 2, C-28,39), 141.2 (quat., × 2, C-33,34), 138.3 (quat., C-12), 132.6 (quat., C-17), 132.3 (quat., C-15), 131.5 (*CH*, C-2), 127.7 (quat., × 2, C-31,36), 127.1 (quat., × 2, C-30,37), 125.2 (quat., × 2, C-29,38), 124.8 (quat., C-13), 120.0 (quat., × 2, C-32,35), 118.8 (CH_2, C-1), 117.6 (quat., C-23), 86.5 (quat., C-18), 67.1 (CH_2, C-26), 66.0 (CH_2, C-3), 54.1 (*CH*, C-7), 47.1 (*CH*, C-27), 43.2 (CH_2, C-21), 41.2 (CH_2, C-5), 40.2 (CH_2, C-10), 30.0 (CH_2, C-9), 28.6 ($CH_3 \times 2$, C-19, 20), 25.2 (CH_2, C-8), 19.4 (CH_3, C-16), 18.0 (CH_3, C-14) and 12.3 (CH_3, C-24); ***m/z*** (ES^+) 768 ($[M+Na]^+$, 100%); **HRMS** *m/z* (ES^+) calcd. for $C_{39}H_{47}N_5NaO_8S$ $[M+Na]^+$ requires 768.3043, found 768.3047.

NH_2-Arg(Pbf)-Gly-OAll, 369



Piperidine (3 mL, 30.4 mmol) was added to a solution of Fmoc-Arg(Pfb)-Gly-OAll **368** (1.5 g, 2.01 mmol) in dry DMF (15 mL) cooled in an ice-bath. After addition, the ice-bath was removed and the solution stirred for 15 min. The solvent was evaporated under reduced pressure to yield a yellow residue. The residue suspended in dichloromethane and adsorbed onto silica gel. Purification by silica gel chromatography eluting with dichloromethane and methanol (100:0 to 90:0) provided *NH_2-Arg(Pbf)-Gly-OAll* **369** (1.0 g, 1.91 mmol, 95%) as a colourless glass: $\boldsymbol{R_f}$ 0.23 (90:10, CH_2Cl_2:MeOH); **m.p.** 48-51 °C; $[\alpha]_D^{20}$ -1.1 (*c* = 1.0, $CHCl_3$); ν_{max} (thin film)/cm^{-1} 3337, 2973, 2932, 1746, 1683, 1651, 1455, 1253, 1105, 1034, 993; **^{1}H NMR** (500 MHz, $CDCl_3$) δ 8.02-7.99 (2H, m, NH × 2, NH-peptide, NH-guanidine), 6.40 (3H, s, *br*, NH_2, N*H*-guanidine, 5.87 (1H, ddt, *J* 17.2, 10.4, 5.7, Hz, C*H*-2), 5.30 (1H, dd, *J* 17.2, 1.4 Hz, C*H*-1-*trans*), 5.22 (1H, dd, *J* 10.4, 1.1 Hz, C*H*-1-*cis*), 4.59 (2H, d, *J* 5.7 Hz, CH_2-3), 4.00 (2H, d, *J* 5.8 Hz, CH_2-5), 3.52 (1H, *J* 7.1 Hz, C*H*-7), 3.23-3.16 (2H, m, CH_2-10), 2.93 (2H, s, CH_2-21), 2.54 (3H, s, CH_2-24), 2.47 (3H, s, CH_2-14), 2.05 (3H, s, CH_2-16), 1.84-1.78 (1H, m, C$H_A$$H_B$-9), 1.65-1.58 (3H, m, C$H_2$-8, C$H_A$$H_B$-9) and 1.43 (6H, s, C$H_2$-19, 20); **^{13}C NMR** (75 MHz, $CDCl_3$) δ 175.5 (quat., C-4) 169.8 (quat., C-6), 158.8 (quat., C-22), 156.5 (quat., C-11), 138.3 (quat., C-12), 132.8 (quat., C-17), 132.2 (quat., C-15), 131.5 (*C*H, C-2), 124.7 (quat., C-13), 118.9 (*C*H_2, C-1), 117.6 (quat., C-23), 86.5 (quat., C-18), 66.0 (*C*H_2, C-3), 54.1 (*C*H, C-7), 43.2 (*C*H_2, C-21), 41.0 (*C*H_2, C-5), 40.6 (*C*H_2, C-10), 31.7 (*C*H_2, C-9), 28.6 (*C*H_3 × 2, C-19, 20), 25.3 (*C*H_2, C-8), 19.3 (*C*H_3, C-16), 18.0 (*C*H_3, C-14) and 12.5 (quat., C-24); ***m/z*** (ES^+) 546 ($[M+Na]^+$, 100%); **HRMS** *m/z* (ES^+) calcd. for $C_{24}H_{37}N_5NaO_6S$ $[M+Na]^+$ requires 546.2362, found 546.2356.

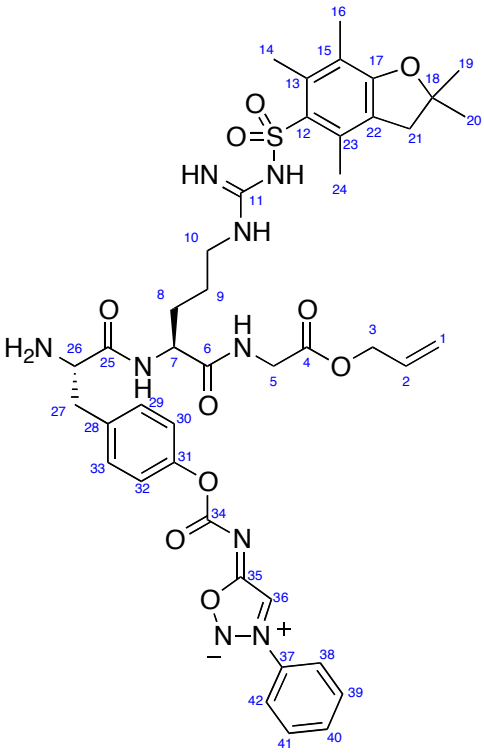
Fmoc-Tyr-(*O*-carbonyl-*N*-3-phenylsydnonimine)-Arg(Pbf)-Gly-OAll, 370



HATU (949 mg, 2.49 mmol) was added to a solution of NH_2-Arg(Pbf)-Gly-OAll **369** (872 mg, 1.66 mmol) and (*S*)-*N*-(((4-(2-(((9*H*-fluoren-9-yl)methoxy)carbonyl)amino)-2-carboxyethyl)phenoxy)carbonyl)-3-phenylsydnonimine **321** (982 mg, 1.66 mmol) in dry dichloromethane (30 mL) cooled to 0 °C. The solution was stirred for 15 min and diisopropylethylamine (distilled, 323 mg, 430 µL, 2.49 mmol) was added. The solution was allowed to warm to room temperature and stirred for 18 h. The resultant solution was diluted with dichloromethane (50 mL) and washed with aq. citric acid (10%, 100 mL), saturated $NaHCO_3$ (100 mL) and brine (20 mL). The organic layer was separated and dried over Na_2SO_4, filtered and the solvent removed under reduced pressure. The resultant residue was uptaken in dichloromethane and adsorbed onto silica gel. Purification by silica gel chromatography eluting with dichloromethane and methanol (100:0, to 95.5:0.5, to 98:2) provided *Fmoc-Tyr-(O-carbonyl-N-3-phenylsydnonimine)-Arg(Pbf)-Gly-OAll* **370** (1.59 g, 1.49 mmol, 87%) as a white solid: $\boldsymbol{R_f}$ 0.57 (90:10, CH_2Cl_2:MeOH); **m.p.** 128-131 °C; $[\alpha]_D^{20}$ +0.9 (*c* = 1.0, $CHCl_3$); ν_{max} (thin film)/cm^{-1} 2361, 1701, 1656, 1580, 1539, 1509, 1470, 1451, 1368, 1276, 1191, 1105, 1033, 1018, 941; **^{1}H NMR** (500 MHz, $CDCl_3$) δ 8.23 (1H, s, C*H*-36), 7.80 (2H, d, *J* 8.2 Hz, C*H*-50,53), 7.69 (2H, d, *J* 7.6 Hz, C*H*-38,42), 7.66 (1H, t, *J* 7.3 Hz, C*H*-40), 7.58 (2H, t, *J* 7.8 Hz, C*H*-39,41), 7.51 (2H, d, *J* 7.2 Hz, C*H*-47,56), 7.32 (2H, t, *J* 7.8 Hz, C*H*-49,54), 7.23 (2H, t, *J* 7.3 Hz, C*H*-48,55), 7.17 (2H, d, *J* 8.1 Hz, C*H*-29,33), 7.07 (2H, d, *J* 8.1 Hz, C*H*-30,32), 6.40

(2H, s, *br*, N*H*-sulfonamide, N*H*-guanidine), 5.99 (1H, s, *br*, N*H*-Fmoc), 5.80 (1H, ddt, *J* 16.9, 10.3, 5.1 Hz, C*H*-2), 5.23 (1H, d, *J* 16.9 Hz, CH_AH$_B$-1-*trans*), 5.15 (1H, d, *J* 10.3 Hz, CH$_A$$H_B$-1-*cis*), 4.48-4.47 (4H, m, CH_2-3, C*H*-7,26), 4.35 (1H, dd, *J* 10.6, 7.6 Hz, CH_AH$_B$-44), 4.19 (1H, dd, *J* 11.6, 7.6 Hz, CH$_A$$H_B$-44), 4.06 (1H, t, *J* 7.6 Hz, C*H*-45), 3.97-3.95 (2H, m, CH_2-10), 3.17-3.12 (2H, m, CH_2-5), 3.07 (2H, d, *J* 4.2 Hz, CH_2-27), 2.87 (2H, s, CH_2-21), 2.52 (3H, s, CH_2-14), 2.46 (3H, s, CH_2-16), 2.02 (3H, s, CH_2-24), 1.90-1.82 (1H, m, CH_AH$_B$-8), 1.68-1.63 (1H, m, CH$_A$$H_B$-8) and 1.46-1.39 (8H, m, CH_2-9, CH_2-19,20); **^{13}C NMR** (75 MHz, CDCl3) δ 175.5 (quat., × 2, C-4,35), 172.0 (quat., C-6), 171.6 (quat., C-25), 169.5 (quat., C-34), 159.8 (quat., C-17), 159.7 (quat., C-11), 156.4 (quat., C-43), 150.8 (quat., C-31), 143.9, 143.7 (quat., × 2, C-46, 57), 141.2 (quat., C-41, 52), 139.7 (quat., C-12), 133.6 (*CH*, C-40), 133.3 (quat., C-37), 133.1 (quat., C-28), 132.8 (quat., C-22), 132.5 (quat., C-15), 131.6 (*CH*, C-2), 130.6 (*C*H × 2, C-39,41), 130.1 (*C*H × 2, C-29,33), 127.7 (*C*H × 2, C-49,54), 127.1 (*C*H × 2, C-48,55), 125.1 (*C*H × 2, C-47,56), 124.8 (quat., C-13), 122.0 (*C*H × 2, C-30,32), 121.7 (*C*H × 2, C-38,42), 119.9 (*C*H × 2, C-50,53), 118.7 (*C*H$_2$, C-1), 117.6 (quat., C-23), 103.8 (*CH*, C-36), 86.5 (quat., C-18), 67.2 (*C*H$_2$, C-44), 65.9 (*C*H$_2$, C-3), 54.3 (*CH*, C-26), 53.5 (*CH*, C-7), 47.0 (*CH*, C-45), 43.2 (*C*H$_2$, C-21), 41.2 (*C*H$_2$, C-5), 40.6 (*C*H$_2$, C-10), 37.7 (*C*H$_2$, C-27), 29.6 (*C*H$_2$, C-8), 28.6 (*C*H$_3$ × 2, C-19, 20), 25.1 (*C*H$_2$, C-9), 19.3 (*C*H$_3$, C-16), 18.0 (*C*H$_3$, C-14) and 12.5 (*C*H$_3$, C-24); ***m/z*** (ES$^+$) 1118 ([M+Na]$^+$, 100%); **HRMS** *m/z* (ES$^+$) calcd. for $C_{57}H_{61}N_9NaO_{12}S$ [M+Na]$^+$ requires 1118.4058, found 1118.4062.

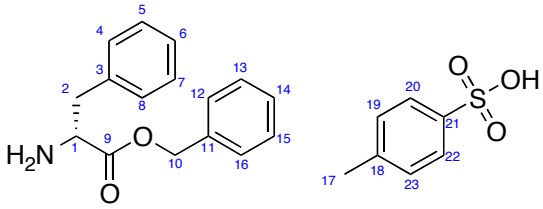
NH_2-Tyr-(*O*-carbonyl-*N*-3-phenylsydnonimine)-Arg(Pbf)-Gly-OAll, 364



Piperidine (3.0 mL, 30.4 mmol) was added to a solution of Fmoc-Tyr-(*O*-carbonyl-*N*-3-phenylsydnonimine)-Arg(Pbf)-Gly-OAll **370** (1.01 g, 0.92 mmol) in dry DMF (15 mL) cooled in an ice-bath. After addition, the ice-bath was removed and the solution stirred for 15 min. The solvent was evaporated under reduced pressure to yield a yellow residue. The residue suspended in dichloromethane and adsorbed onto silica gel. Purification by silica gel chromatography, eluting with dichloromethane and methanol (100:0 to 90:10), provided *NH_2-Tyr-(O-carbonyl-N-3-phenylsydnonimine)-Arg(Pbf)-Gly-OAll* **364** (725 mg, 0.83 mmol, 82%) as a yellow crystalline solid; $\boldsymbol{R_f}$ 0.31 (90:10, CH_2Cl_2:MeOH); **m.p.** 103-107 °C; $[\boldsymbol{\alpha}]_{\mathbf{D}}^{\mathbf{20}}$ +13.3 = 1.0, $CHCl_3$); $\boldsymbol{\nu_{max}}$ (thin film)/cm^{-1} 2928, 2926, 2854, 1745, 1650, 1583, 1558, 1508, 1471, 1408, 1369, 1283, 1193, 1106, 975, 852; **^{1}H NMR** (500 MHz, CDCl3) δ 8.41 (1H, s, C*H*-36), 8.02-7.83 (4H, m, NH_2, C*H*-38, 42), 7.70-7.61 (4H, m, N*H*-Arg-Gly, C*H*-39,40,41), 7.17 (2H, d, *J* 7.2 Hz, CH-29,33), 7.06 (2H, d, *J* 7.2 Hz, C*H*-30,32), 6.48 (2H, s, *br*, N*H*-sulfonamide, N*H*-guanidine), 5.83 (1H, ddt, *J* 17.1, 10.3, 5.0 Hz, C*H*-2), 5.27 (1H, d, *J* 17.1 Hz, CH_AH_B-1-*trans*), 5.19 (1H, d, *J* 10.3 Hz, CH_AH_B-1-*cis*), 4.56-4.50 (3H, m, 3H, CH_2-3, C*H*-7), 4.01-3.91 (2H, m, CH_2-5), 3.76-3.71 (1H, m, C*H*-26), 3.21-3.15 (2H, m, CH_2-10), 2.94-2.90 (4H, m, CH_2-27, CH_2-21), 2.54 (3H, s, C*H*-14), 2.47 (3H, s, CH_2-16), 2.05 (3H, s, CH_2-24), 1.89-1.84 (1H, m, CH_AH_B-8), 1.70-1.64 (1H, m, CH_AH_B-8) and 1.46-1.41 (8H, m, CH_2-9, CH_2-19,20); **^{13}C NMR** (125 MHz, $CDCl_3$) 175.5 (quat., C-35), 172.3 (quat., C-4), 170.3 (quat., × 2, C-6,25), 169.5 (quat., C-34),

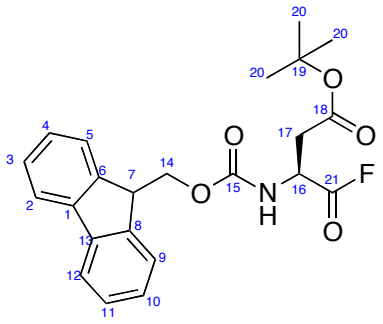
158.7 (quat., C-17), 156.5 (quat., C-11), 150.8 (quat., C-31), 138.8 (quat., C-12), 133.7 (quat., C-37), 133.3 (*CH*, C-40), 133.1 (quat., C-28), 132.8 (quat., C-22), 132.3 (quat., C-15), 131.6 (*CH*, C-2), 130.6 (*C*H × 4, C-29,33,38,42), 124.7 (quat., C-13), 122.1 (*C*H × 2, C-30,32), 121.6 (*C*H × 2, C-39,41), 118.8 (CH_2, C-1), 117.5 (quat., C-23), 104.0 (*CH*, C-46), 86.4 (quat., C-18), 65.9 (CH_2, C-3), 55.7 (*CH*, C-26), 52.6 (*CH*, C-7), 43.2 (CH_2, C-21), 42.0 (CH_2, C-5), 40.5 (CH_2, C-27), 41.2 (CH_2, C-10), 29.8 (CH_2, C-8), 28.6 (CH_3 × 2, C-19, 20), 25.1 (CH_2, C-9), 19.3 (CH_3, C-16), 18.0 (CH_3, C-14) and 12.5 (CH_3, C-24); ***m/z*** (ES^+) 896 ($[M+Na]^+$, 100%); **HRMS** *m/z* (ES^+) calcd. for $C_{42}H_{11}N_9NaO_{10}S$ $[M+Na]^+$ requires 896.3372, found 896.3373.

NH_2-D-Phe-OBn *p*-toluenesulfonate, 371[378]



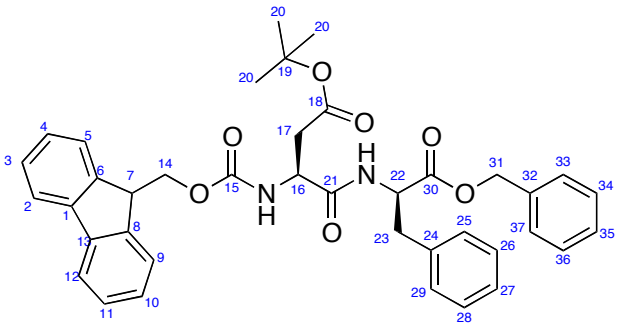
p-Toluenesulfonic acid (6.9 g, 36.1 mmol) was added to a solution of D-phenylalanine **371** (5.0 g, 30.4 mmol) in toluene (50 mL) and benzyl alcohol (65 mL). The mixture was heated to reflux using a Dean-Stark trap overnight. The resultant solution was cooled to room temperature and then in an ice-bath and diethyl ether added. The precipitant was collected to recrystallised from toluene to afford NH_2-D-Phe-OBn *p*-toluenesulfonate **372** (10.8 g, 25 mmol, 81%) as white waxy crystals: **m.p.** 120-121 °C (toluene); $[\alpha]_D^{20}$-7.1, (*c* = 1.0, MeOH [*S* enantiomer, Lit.[421] +7.2, (*c* = 1.0, MeOH]; **^{1}H NMR** (500 MHz, $CDCl_3$) δ 8.49 (3H, s, *br*, N*H*$_2$.TosO*H*), 7.51 (2H, d, *J* 8.0 Hz, C*H*-19,23), 7.36-7.10 (10H, m, C*H*-4,5,6,7,8,12,13,14,15,16), 7.13 (2H, d, *J* 8.0 Hz, C*H*-20,22), 5.13 (2H, s, CH_2-10), 4.38 (1H, t, *J* 6.7 Hz, C*H*-1), 3.16 (1H, dd, *J* 14.1, 6.0 Hz, CH_AH$_B$-2), 3.06 (1H, dd, *J* 13.8, 7.5 Hz, CH$_A$$H_B$-2), 2.85 (3H, s, C$H_2$-17); **^{13}C NMR** (125 MHz, $CDCl_3$) δ 169.4 (quat., C-9), 138.8 (quat., C-18), 138.3 (quat., C-21), 135.3 (*CH*, C-14), 134.9 (*CH*, C-6), 129.1, 128.9, 128.6, 128.0 (*C*H × 6, C-4,5,8,7,19,23), 128.5 (*C*H × 2, C*H*-20,22), 127.9 (quat., C-3), 127.7 (quat., C-11), 126.0 (*C*H × 2, C*H*-19,23), 67.6 (CH_2, C-10), 53.7 (*CH*, C-1), 36.5 (CH_2, C-2) and 21.3 (CH_3, C-17); ***m/z*** 279 ($[M+Na]^+$, 100%). The data were in agreement with the literature values.[378]

Fmoc-Asp(OtBu)-F, 374[379]



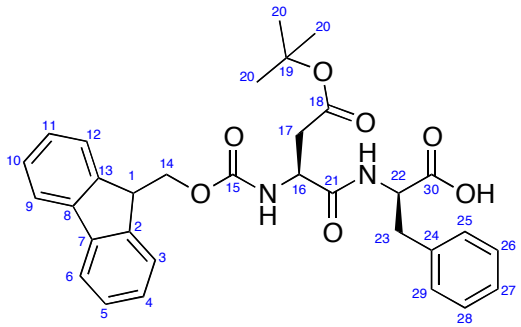
Cyanuric fluoride (656 mg, 420 µL. 4.86 mmol) was added to a solution of Fmoc-Asp(OtBu)-OH **373** (1.00 g, 2.43 mmol) in dry dichloromethane (13 mL). To this was added pyridine (200 µL, 2.43 mmol) and the solution was stirred for 3 h. The resulting suspension was diluted with dichloromethane (10 mL) and washed with ice-cold water (20 mL × 2), dried over $MgSO_4$. The mixture was filtered and the solvent removed under reduced pressure to give Fmoc-Asp(OtBu)-F **374** (940 mg, 2.28 mmol, 94%) as a pale pink solid, which was used without any further purification: **m.p.** 74-76 °C, [Lit.[379] 74-75 °C]; $[\alpha]_D^{20}$ +3.9 (*c* = 0.5, EtOAc, [Lit.[379] +4.0 (*c* = 0.5, EtOAc]; **^{1}H NMR** (500 MHz, $CDCl_3$) δ 7.77 (2H, d, *J* 7.6 Hz, C*H*-2,12), 7.59 (2H, d, *J* 7.4 Hz, C*H*-5,9), 7.41 (2H, t, *J* 7.4 Hz, C*H*-3,11), 7.32 (2H, t, *J* 7.4 Hz, C*H*-4,10), 5.72 (1H, N*H*-Fmoc), 4.83 (1H, ddd, *J* 12.0, 8.0, 4.0 Hz, C*H*-16), 4.48 (1H, dd, *J* 10.6, 7.2 Hz, CH_AH_B-14), 4.39 (1H, dd, *J* 10.5, 7.3 Hz, CH_AH_B-14), 4.25 (1H, t, *J* 7.0 Hz, C*H*-7), 3.00 (1H, dd, *J* 17.7, 4.7 Hz, CH_AH_B-17), 2.82 (1H, dd, *J* 17.7, 4.2 Hz, CH_AH_B-17) and 1.49 (9H, s, CH_3 × 3, CH_2-20); **^{13}C NMR** (125 MHz, $CDCl_3$) δ 169.6 (quat., C-18), 161.5 (quat., d, *J* 366.7 Hz, C-21), 155.4 (quat., C-15), 143.6, 143.5 (quat., × 2, C-6,8), 141.3 (quat., × 2, C-1,13), 127.4 (*C*H × 2, C-3,11), 125.4 (*C*H × 2, C-4,10), 120.1 (*C*H × 2, C-2,12), 83.0 (quat., C-19), 67.6 (CH_2, C-14), 49.4 (*CH*, d, *J* 60.8 Hz, C-16), 47.0 (*CH*, C-7), 37.3 (CH_2, C-17) and 28.0 (CH_3 × 3, CH_2-20); **^{19}F NMR** (376 MHz, $CDCl_3$) δ 28.2 (s, CO*F*); ***m/z*** (ES^+) 414 ($[M+H]^+$, 100%). The data were in agreement with the literature values.[379]

Fmoc-Asp(OtBu)-D-Phe-OBn, 375[379]



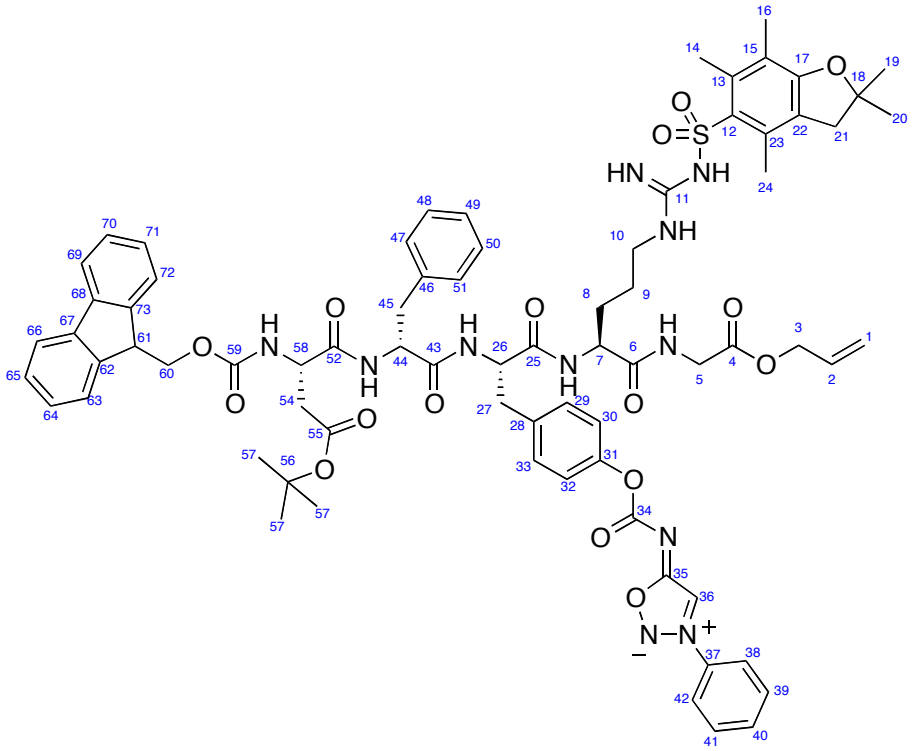
Fmoc-Asp(OtBu)-F **374** (940 mg, 2.28 mmol in dry CH_2Cl_2, 10 mL) was added dropwise to a solution of NH_2-D-Phe-OBn *p*-toluenesulfonate **372** (1.07 g, 2.51 mmol) and diispropylethylamine (distilled, 657 mg, 870 µL, 5.01 mmol) in dry dichloromethane (20 mL) cooled to -20 °C and stirred for 1 hour. The solution as diluted with dichloromethane (20 mL) and washed with saturated sodium bicarbonate (30 mL), brine (30 mL), dried over $MgSO_4$. The mixture was filtered and the solvent evaporated under reduced pressure to give an off-white residue. The residue was uptaken in dichloromethane and adsorbed onto silica gel. Purification by silica gel chromatography eluting with ethyl acetate and hexanes (5:95) provided Fmoc-Asp(OtBu)-D-Phe-OBn **375** (1.00 g, 1.54 mmol, 68%) as a white crystalline solid: **m.p.** 79-81 °C; $\boldsymbol{R_f}$ 0.85 (50:50, EtOAc:PE); $\boldsymbol{[\alpha]_D^{20}}$ +10.1 (*c* = 1.0, $CHCl_3$); **^{1}H NMR** (500 MHz, $CDCl_3$) δ 7.78 (2H, d, *J* 7.6 Hz, C*H*-2,12), 7.59-7.56 (2H, m, C*H*-5,9), 7.41 (2H, t, *J* 7.4 Hz, C*H*-3,11), 7.36-7.27 (7H, m, C*H*-4,10,25,26,27,28,29), 7.21-7.16 (3H, m, C*H*-33,37, 35), 7.08-7.02 (3H, m, C*H*-34, 36, N*H*-Asp-Phe), 5.98 (2H, d, *J* 8.3, N*H*-Fmoc), 5.16 (1H, d, *J* 12.1 Hz, CH_AH_B-31), 5.11 (1H, d, *J* 12.1 Hz, CH_AH_B-31), 4.91-4.85 (1H, m, C*H*-22), 4.58-4.53 (1H, m, C*H*-16), 4.41-4.32 (2H, m, CH_2-14), 4.21 (1H, dd, *J* 12.8, 6.3 Hz, C*H*-7), 3.17-3.06 (2H, m, CH_2-23), 2.89-2.84 (1H, m, CH_AH_B-17), 2.61-2.55 (1H, m, CH_AH_B-17) and 1.45 (9H, app, d, CH_3 × 3, CH_3-20, major and minor rotamer); **^{13}C NMR** (125 MHz, $CDCl_3$) δ 171.3 (quat., C-30), 170.9 (quat., C-21), 170.2 (quat., C-18), 156.0 (quat., C-15), 143.8, 143.7 (quat., × 2, C-6,8), 141.3 (quat., × 2, C-1,13), 135.3 (*CH*, C-35), 135.1 (quat., C-32), 129.3 (*C*H × 4, C-26, 28, 34, 36), 128.6 (*C*H × 5 and quat., C-24,25,29,27,33,37), 127.8 (*C*H × 2, C-3,11), 127.1 (*C*H × 2, C-4,10), 125.2 (*C*H × 2, C-5,9), 120.1 (*C*H × 2, C-2,12), 82.0 (quat., C-19), 67.4 (CH_2, C-31), 67.3 (CH_2, C-14), 53.5 (*CH*, C-16), 51.0 (*CH*, C-22), 47.1 (*CH*, C-7), 37.8 (CH_2, C-23), 37.4 (CH_2, C-17) and 28.0 (CH_3 × 3, C-20 × 3); ***m/z*** (ES^+) 671 ($[M+Na]^+$, 100%). The data were in agreement with the literature values.[380]

Fmoc-Asp(OtBu)-D-Phe-OH, 365[380]



Palladium on carbon (5%, 363 mg) was added to a solution of Fmoc-Asp(OtBu)-D-Phe-OBn **375** (3.63 g, 5.60 mmol) and ammonium formate (1.06 g, 16.8 mmol) in THF:MeOH (1:1, 40 mL) cooled in an ice-bath. The resulting suspension was stirred for 30 min. The resultant suspension was filtered through Celite and evaporated under reduced pressure to give a colourless residue. This was uptaken in dichloromethane and adsorbed onto silica gel. Purification by silica gel chromatography eluting with dichloromethane and methanol (90:10) provided Fmoc-Asp(OtBu)-D-Phe-OH **365** (2.81 g, 4.76 mmol, 85%) as a white crystalline solid as a mixture of rotamers: $\boldsymbol{R_f}$ 0.23 (50:50, EtOAc:PE)**; m.p.** 160-162 °C [Lit.[380] 159-162 °C, Carpino *et al.*[380]); $[\boldsymbol{\alpha}]_{\mathbf{D}}^{\mathbf{20}}$ -5.5, (*c* = 1.0, $CHCl_3$); **^{1}H NMR** (500 MHz, d_6-DMSO) δ 8.14 (1H, d, *J* 8.0, N*H*-Fmoc, minor rotamer), 7.94 (1H, d, *J* 7.9, N*H*-Fmoc, major rotamer), 7.89 (2H, d, *J* 7.3 Hz, C*H*-6,9), 7.70 (2H, t, *J* 6.7 Hz, C*H*-3,12), 7.62 (1H, d, *J* 8.6, N*H*-peptide, major rotamer), 7.57 (1H, d, *J* 8.6, N*H*-peptide, minor rotamer), 7.41 (2H, d, *J* 7.3 Hz, C*H*-3,12), 7.31 (2H, t *J* 7.3 Hz, C*H*-4, 11), 7.26-7.15 (5H, m, C*H*-25,26,27,28,29), 4.45-4.36 (2H, m, C*H*-16,22), 4.32-4.20 (3H, m, CH_2-14, C*H*-1), 3.08-3.02 (1H, m, CH_AH_B-23), 2.91 (1H, dd, *J* 8.6, *J* 13.8 Hz, CH_AH_B-23), 2.61 (1H, dd, *J* 15.9, 4.2 Hz, CH_AH_B-17), 2.41 (1H, dd, *J* 16.6, 9.0 Hz, CH_AH_B-17) and 1.36-1.35 (9H, m, CH_2-20, m*a*jor and minor rotamer.); **^{13}C NMR** (125 MHz, d_6-DMSO) δ 173.2 (quat., C-30), 171.2 (quat., C-21), 169.7 (quat., C-18), 156.2 (quat., C-15) 144.3, 144.2 (quat., × 2, C-6,8), 141.2 (quat. × 2, C-1,13), 137.8 (quat., C-24), 129.7 (*C*H × 2, C-26,28), 128.7 (*C*H × 2, C-25, 29), 128.2 (*CH*, C-27), 127.6 (*C*H × 2, C-3,11), 126.9 (*C*H × 2, C-4,10), 125.8 (*C*H × 2, C-5,9), 120.6 (*C*H × 2, C-2,12), 80.6 (quat., C-19), 66.3 (CH_2, C-14), 53.9 (*CH*, C-16), 51.8 (*CH*, C-22), 47.0 (*CH*, C-7), 38.0 (CH_2, C-23), 37.1 (CH_2, C-17) and 28.2 (CH_3 × 3, C-20 × 3); ***m/z*** (ES^+) 557 ($[M-H]^+$, 100%). The data were in agreement with the literature values.[380]

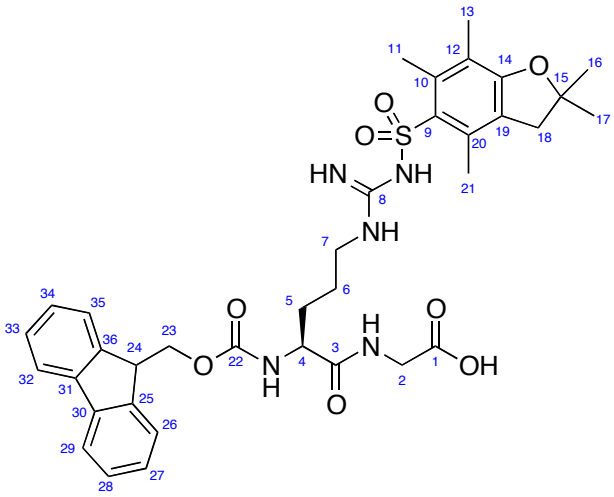
Fmoc-Asp(OtBu)-D-Phe-Tyr-(*O*-carbonyl-*N*-3-phenylsydnonimine)-Arg(Pbf)-Gly-OAll, 376



HATU (431 mg, 1.13 mmol) was added to a solution of NH_2-Tyr-(*O*-carbonyl-*N*-3-phenylsydnonimine)-Arg(Pbf)-Gly-OAll **374** (660 mg. 0.756 mmol) and Fmoc-Asp(OtBu)-D-Phe-OH **375** (422 mg, 0.756 mmol) in dry dichloromethane (50 mL) cooled to 0 °C. The solution was stirred for 15 min and diisopropylethylamine (distilled, 147 mg, 200 µL, 1.13 mmol) was added. The solution was allowed to warm to room temperature and stirred for 18 h. The resultant solution was diluted with dichloromethane (50 mL) and washed with aq. citric acid (10%, 50 mL), saturated $NaHCO_3$ (50 mL) and brine (50 mL). The organic layer was separated and dried over Na_2SO_4, filtered and the solvent removed under reduced pressure. The resultant residue was uptaken in dichloromethane and adsorbed onto silica gel. Purification by silica gel chromatography eluting with dichloromethane and methanol (100:0, to 97:3) provided *Fmoc-Asp(OtBu)-D-Phe-Tyr(O-carbonyl-N-3-phenylsydnonimine)-Arg(Pbf)-Gly-OAll* **376** (880 mg, 0.622 mmol, 82%) as a pale yellow solid: $\boldsymbol{R_f}$ 0.78 (90:10, CH_2Cl_2:MeOH); **m.p.** 138-143 °C; $[\boldsymbol{\alpha}]_{\mathbf{D}}^{\mathbf{20}}$-20.1, (*c* = 1.0, $CHCl_3$); $\boldsymbol{\nu_{max}}$ (thin film)/cm^{-1} 2926, 2852, 1729, 1884, 1654, 1651, 1635, 1586, 1537, 1508, 1471, 1367, 1280, 1212, 1191, 1168, 1106, 1033, 971; **^{1}H NMR** (500 MHz, $CDCl_3$) δ 8.18-8.17 (1H, m, C*H*-36), 7.80-7.78 (2H, m, C*H*-38,42), 7.75-7.72 (2H, m, C*H*-65,68), 7.71-7.67 (1H, m, C*H*-40), 7.64-7.61 (2H, m, C*H*-39,41), 7.59-7.55 (2H, m, C*H*-62,71), 7.39-7.36 (2H, m, C*H*-64,69), 7.31-7.28 (2H, m, C*H*-63,70), 7.26-7.15 (4H, m, C*H*-47,48,50,51), 7.13-7.03 (5H, m, C*H*-29,30,32,33,49), 6.34-6.30 (2H, m, 2 × N*H*-guanidine), 5.90-5.71 (1H, m, C*H*-2), 5.30 (1H, d, *J* 17.2 Hz, C*H*-1-*trans*), 5.21 (1H, d, *J* 12.6 Hz, C*H*-1-

cis), 4.61-4.47 (4H, m, CH_2-3, C*H*-26,53), 4.42-4.37 (2H, m, C*H*-7,44), 4.35-4.28 (2H, m, CH_2-59), 4.21-4.11 (3H, m, CH_2-5, C*H*-60), 3.87 (1H, dd, *J* 13.5, 5.0 Hz, CH_AH_B-10), 3.79 (1H, dd, *J* 15.7, 6.0 Hz, CH_AH_B-10), 3.24-3.15 (2H, m, CH_2-17), 3.10-2.88 (6H, m, CH_2-21,45,54), 2.57-2.55 (3H, m, CH_3-14), 2.51-2.49 (3H, m, CH_3-16), 2.06 (3H, s, CH_3-24), 1.93-1.85 (2H, m, CH_2-9) and 1.56-1.38 (17H, m, CH_2-8, CH_3-19,20, 3 × CH_3-57); **^{13}C NMR** (125 MHz, $CDCl_3$) δ 175.7 (quat., C-35), 175.6 (quat., C-4), 172.1 (quat., C-53), 171.4 (quat., C-6), 171.3 (quat., C-25), 170.9 (quat., C-43), 170.2 (quat., C-50), 160.1 (quat., C-17), 159.6 (quat., C-34), 158.7 (quat., C-11), 156.9 (quat., C-58), 151.0 (quat., C-31), 143.7, 143.6 (quat. × 2, C-61,72), 141.1 (quat. × 2, C-66,67), 138.4 (quat., C-12), 133.7 (quat., C-37), 133.3 (*C*H, C-40), 133.2 (quat., C-28), 132.4 (quat. × 2, C-15,22), 131.7 (quat., C-46), 131.4 (*C*H, C-2), 130.7 (*C*H × 2, C-39,41), 130.1 (*C*H × 2, C-29,33), 129.7 (*C*H, C-49), 129.2 (*C*H × 2, C-47,51), 128.8 (*C*H × 2, C-47,51), 127.8 (quat. × 2, C-64,69), 127.1 (quat. × 2, C-63,70), 125.2 (quat. × 2, C-62,71), 123.6 (quat., C-13), 122.2 (*C*H × 2, C-30,32), 121.7 (*C*H × 2, C-38,42), 120.0 (quat. × 2, C-65,68), 118.8 (*C*H_2, C-1), 117.5 (quat., C-23) 103.6 (*C*H, C-36), 86.4 (quat., C-18), 82.0 (quat., C-54); 67.4 (*C*H_2, C-59), 66.0 (*C*H_3, C-3), 55.9 (*C*H, C-26), 55.7 (*C*H, C-51), 53.1 (*C*H, C-7), 50.5 (*C*H, C-44), 46.9 (*C*H, C-60), 43.1 (*C*H_3, C-21), 41.3 (*C*H_2, C-5), 40.8 (*C*H_3, C-10), 40.7 (*C*H_2, C-27), 36.2 (*C*H_2, C-45), 35.9 (*C*H_2, C-52), 28.6 (*C*H3 × 2, C-19,20), 28.2 (*C*H_2, C-8), 28.1 (*C*H_3 × 3, C-28), 25.4 (*C*H_2, C-9), 19.3 (*C*H_3, C-16) 18.0 (*C*H_3, C-14) and 12.5 (*C*H_3, C-24); ***m/z*** (ES^+) 1414 ($[M+H]^+$, 100%); **HRMS** *m/z* (ES^+) calcd. for $C_{74}H_{84}N_{11}O_{16}S$ $[M+H]^+$ requires 1414.5813, found 1414.5780.

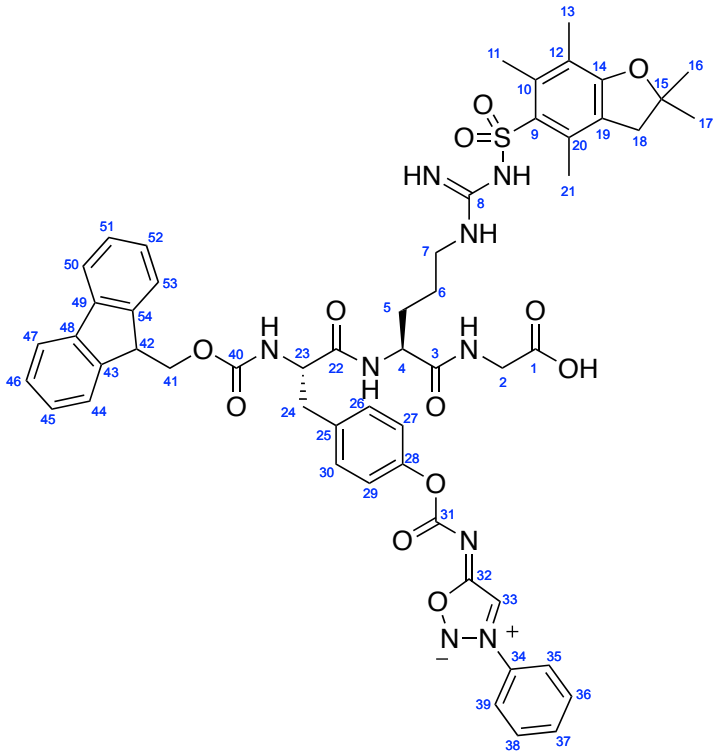
Fmoc-Arg(Pbf)-Gly-OH, 377



Palladium tetrakis(triphenylphosphine) (16 mg, 0.013 mmol, 5 mol%) and dimedone (94 mg, 0.670 mmol) was added to a solution of Fmoc-Arg(Pbf)-Gly-OAll **368** (200 mg, 0.268 mmol) in dry THF (15 mL), and the solution stirred at room temperature for 3 h. Dichloromethane (20 mL) was added and the solution was washed with aq. HCl (2 N, 4 mL) and brine (5 mL), dried over $MgSO_4$, filtered and evaporated. The resultant residue was uptaken in dichloromethane and

adsorbed onto silica gel. Purification by silica gel chromatography, eluting with dichloromethane, methanol and acetic acid (98:2, 95:5:1), furnished *Fmoc-Arg(Pbf)-Gly-OH* **377** (180 g, 0.255 mmol, 95%) as a white solid: $\boldsymbol{R_f}$ 0.54 (90:10:0.5, CH_2Cl_2:MeOH:AcOH); **m.p.** 128-131 °C; $[\alpha]_D^{20}$ -3.2, (*c* = 1.0, $CHCl_3$, MeOH; ν_{max} (thin film)/cm^{-1} 2926, 2854, 2528, 1719, 1684, 1669, 1653, 1621, 1560, 1451, 1407, 1370, 1250, 1105, 1034, 994; **^{1}H NMR** (500 MHz, d6-DMSO) δ 8.21 (1H, t, *J* 5.4, N*H*-Arg-Gly), 7.90 (2H, d, *J* 7.6 Hz, C*H*-29,32), 7.75 (2H, t, *J* 6.8 Hz, C*H*-26,35), 7.56 (1H, d, *J* 8.4, N*H*-Fmoc), 7.43 (2H t, *J* 6.8 Hz, C*H*-28,33), 7.34 (2H, t, *J* 6.4 Hz, C*H*-27,34), 6.78 (1H, s, N*H*-sulfonamide), 6.44 (1H, s, N*H*-guanidine), 4.32-4.21 (3H, m, CH_2-23, C*H*-24), 4.04 (1H, dd, *J* 13.5, 8.6 Hz, C*H*-4), 3.80 (1H, dd, *J* 17.5, 5.8 Hz, CH_AH$_B$-2), 3.74 (1H, dd, *J* 5.6, *J* 17.5 Hz, CH$_A$$H_B$-2), 3.06 (2H, dd, *J* 12.3, 6.7 Hz, CH_2-7), 2.95 (2H, s, CH_2-18), 2.52 (3H, s, CH_2-21), 2.44 (3H, s, CH_2-11), 2.02 (3H, s, CH_2-13), 1.72-1.66 (1H, m, CH_AH$_B$-5) and 1.54-1.39 (9H, m, CH$_A$$H_B$-5, C$H_2$-9, C$H_2$-16,17); **^{13}C NMR** (125 MHz, d_6-DMSO) δ 173.0 (quat., C-1), 171.6 (quat., C-3), 157.8 (quat., C-14), 156.6 (quat., C-8), 156.4 (quat., C-22), 144.4, 144.2 (quat., × 2, C-25,36), 137.8 (quat., C-9), 134.8 (quat., C-19), 131.9 (quat., C-12), 131.1 (quat., × 2, C-30,31), 128.1 (quat., × 2, C-28,33) 127.6 (quat., × 2, C-27,34), 125.8 (quat., × 2, C-26, 35), 124.8 (quat., C-10), 120.6 (quat., × 2, C-29, 23), 116.8 (quat., C-20), 86.8 (quat., C-15), 66.1 (*C*H$_2$, C-23), 54.6 (*CH*, C-4), 41.2 (*C*H$_2$, C-2), 40.4 (*C*H$_2$, C-7), 29.8 (*C*H$_2$, C-5), 28.7 (*C*H$_3$ × 2, C-16,17), 26.0 (*C*H$_2$, C-6), 19.5 (*C*H$_3$, C-13), 18.1 (*C*H$_3$, C-11), 12.8 (*C*H$_3$, C-21), 47.1 (*CH*, C-24), 42.8 (*C*H$_2$, C-18); ***m/z*** (ES$^-$) 704 ([M-H]$^-$, 100%); **HRMS** *m/z* (ES$^+$) calcd. for $C_{36}H_{42}N_5O_8S$ [M+H]$^+$ requires 704.2760, found 704.2766.

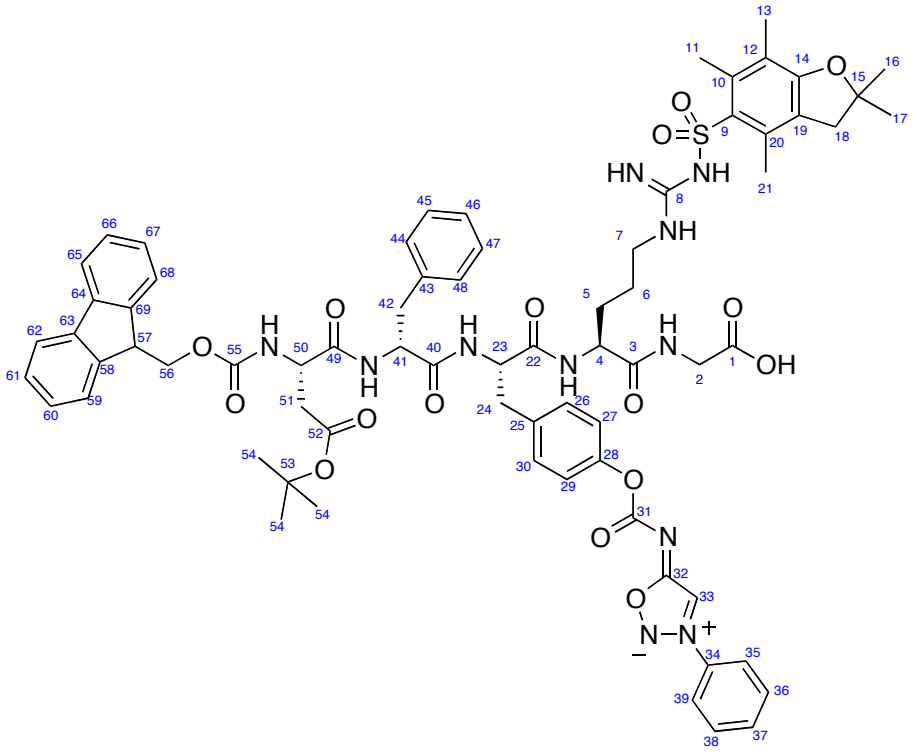
Fmoc-Tyr(*O*-carbonyl-*N*-3-phenylsydnonimine)-Arg(Pbf)-Gly-OH, 378



Palladium tetrakis(triphenylphosphine) (4 mg, 0.004 mmol, 6.7 mol%) and dimedone (64 mg, 0.46 mmol) was added to a solution of Fmoc-Tyr(*O*-carbonyl-*N*-3-phenylsydnonimine)-Arg(Pbf)-Gly-OAll **370** (100 mg, 0.09 mmol) in dry THF (10 mL), and the solution stirred at room temperature for 3 h. Dichloromethane (20 mL) was added and the solution was washed with aq. HCl (2 N, 10 mL) and brine (10 mL), dried over $MgSO_4$, filtered and evaporated. The resultant residue was uptaken in dichloromethane and adsorbed onto silica gel. Purification by silica gel chromatography, eluting with dichloromethane, methanol and acetic acid (98:2, 95:5:1), furnished *Fmoc-Arg(Pbf)-Gly-OH* **378** (85 g, 0.08 mmol, 88%) as a pale yellow solid. $\boldsymbol{R_f}$ 0.60 (90:10:0.5, CH_2Cl_2:MeOH:AcOH); **m.p.** 195-200 °C; $[\boldsymbol{\alpha}]_{\mathbf{D}}^{\mathbf{20}}$ -7.9, (*c* = 1.0, $CHCl_3$, MeOH; $\boldsymbol{\nu_{max}}$ (thin film)/cm^{-1} 3347, 2977, 2932, 1730, 1652, 1584, 1508, 1471, 1368, 1285, 1192, 1166, 1107, 1018, 974, 846; **^{1}H NMR** (500 MHz, d_6-DMSO) δ 8.57 (1H, s, C*H*-33), 8.19 (1H, d, *J* 6.5 Hz, N*H*-Fmoc), 8.10 (1H, s, *br*, N*H*-Tyr-Arg), 8.05 (2H, d, *J* 7.8 Hz, C*H*-25,39), 7.85 (2H, d, *J* 7.6 Hz, C*H*-47,50), 7.77 (1H, t, *J* 7.1 Hz, C*H*-37), 7.72 (2H, t, *J* 7.6 Hz, C*H*-36,38), 7.66-7.63 (3H, m, C*H*-44,53, N*H*-Arg-Gly), 7.40-7.36 (2H, m, C*H*-46,51), 7.32-7.26 (4H, m, C*H*-26,30,45,52), 7.07 (1H, t, *J* 7.4 Hz, N*H*-guanidine), 7.01 (2H, d, *J* 8.1, C*H*-27,29), 6.51 (2H, s, *br*, N*H*-guanidine, N*H*-sulfonamide), 4.35-4.26 (2H, m, C*H*-4,23), 4.20-4.11 (3H, m, C*H*-41,42), 3.72-3.63 (2H, m, CH_2-2), 3.06-3.01 (3H, m, CH_2-7, CH_AH_B-24), 3.92 (2H, s, CH_2-18), 2.80-2.75 (1H, m, CH_AH_B-24), 2.57 (3H, s, CH_3-21), 2.41 (3H, s, CH_3-11), 1.98 (3H,

s, C*H*$_3$-13), 1.73-1.68 (1H, m, C*H*$_A$H$_B$-5), 1.57-1.60 (1H, m, CH$_A$*H*$_B$-5) and 1.46-1.37 (8H, m, C*H*$_2$-6, C*H*$_3$-16,17); **^{13}C NMR** (125 MHz, d$_6$-DMSO) δ 175.4 (quat., C-32), 172.5 (quat., C-1), 171.8 (quat., C-3), 171.9 (quat., C-22), 157.8 (quat., C-31), 157.9 (quat., C-14), 156.6 (quat., C-8), 156.2 (quat., C-40), 150.9 (quat., C-28), 144.3, 144.2 (quat. × 2, C-43,54), 141.1 (quat. × 2, C-48,49), 137.7 (quat., C-9), 134.6 (quat., C-19), 134.4 (quat., C-34), 134.1 (quat., C-25), 133.5 (*C*H, C-38), 131.9 (quat., C-12), 130.7 (*C*H × 2, C-36,38), 130.6 (*C*H × 2, C-27,39), 128.1 (*C*H × 2, C-46,51), 127.5 (*C*H × 2, C-45,52), 125.8, 125.7 (*C*H × 2, C-44 53), 124.8 (quat., C-10), 122.9 (*C*H × 2, C-35,39), 121.7 (*C*H × 2, C-26,30), 120.5 (*C*H × 2, C-47,50), 116.7 (quat., C-20), 105.2 (*C*H, C-33), 86.7 (quat, C-15), 66.1 (*C*H$_2$, C-41), 56.6 (*C*H, C-23), 55.4 (*C*H, C-4), 47.0 (*C*H, C-42), 42.9 (*C*H$_2$, C-18), 41.9 (*C*H$_2$, C-2), 40.1 (*C*H$_2$, C-7), 37.2 (*C*H$_2$, C-24), 30.0 (*C*H$_2$, C-5), 28.7 (*C*H3 × 2, C-16, 17), 25.6 (*C*H$_2$, C-6), 19.4 (*C*H$_3$, C-13), 18.0 (*C*H$_3$, C-11) and 12.7 (*C*H$_3$, C-21); ***m/z*** (ES$^-$) 1054 ([M-H]$^-$, 100%); **HRMS** *m/z* (ES$^-$) calcd. for $C_{54}H_{56}N_9O_{12}S$ [M-H]$^-$ requires 1054.3775, found 1054.3772.

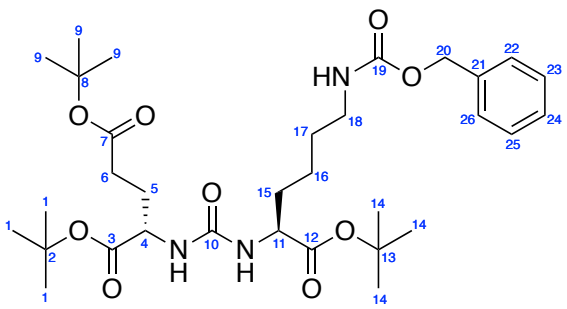
Fmoc-Asp(OtBu)-D-Phe-Tyr-(*O*-carbonyl-*N*-3-phenylsydnonimine)-Arg(Pbf)-Gly-OH, 379



Palladium tetrakis(triphenylphosphine) (2 mg, 0.0018 mmol, 5 mol%) and dimedone (25 mg, 0.176 mmol) was added to a solution of **376** (50 mg, 0.035 mmol) in dry THF (3 mL), and the solution stirred at room temperature for 3 h. Dichloromethane (20 mL) was added and the solution was washed with aq. HCl (2 N, 4 mL) and brine (5 mL), dried over MgSO$_4$, filtered and

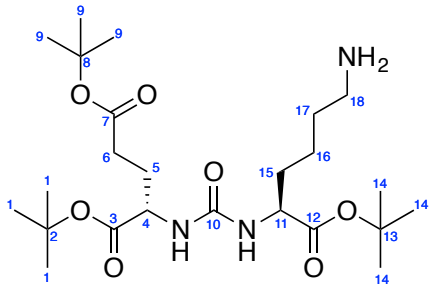
evaporated. The resultant residue was uptaken in dichloromethane and adsorbed onto silica gel. Purification by silica gel chromatography eluting with dichloromethane, methanol and acetic acid (100:0:0, to 97:3:0 to 95:5:1) provided *Fmoc-Asp*(*OtBu)-D-Phe-Tyr*-(*O-carbonyl-N-3-phenylsydnonimine)-Arg*(*Pbf)-Gly-OH* **379** (37 mg, 0.027 mmol, 77%) as a pale yellow solid: $\boldsymbol{R_f}$ 0.54 (90:10:1, CH_2Cl_2:MeOH:AcOH); **m.p.** 159-165 °C; $[\boldsymbol{\alpha}]_{\boldsymbol{D}}^{\boldsymbol{20}}$+9.2 (*c* = 0.75, MeOH; $\boldsymbol{\nu_{max}}$ (thin film)/cm^{-1} 2362, 2342, 2335, 1701, 1696, 1688, 1654, 1559, 1561, 1512, 1464, 1274, 1267, 1261, 764; **^{1}H NMR** (500 MHz, d_6-DMSO) δ 8.62-8.57 (1H, m, C*H*-33), 1.44 (1H, d, *J* 9.2 Hz, N*H*-amide), 8.31 (1H, d, *J* 9.0 Hz, N*H*-amide), 8.22 -8.13 (1H, m, N*H*-Fmoc), 8.06 (2H, d, *J* 7.1 Hz, C*H*-35, 39), 8.00-7.83 (3H, m, C*H*-62, 65, N*H*-amide), 7.78 (1H, t, *J* 7.6 Hz, C*H*-37), 7.74-7.66 (4H, m, C*H*-36,38,59,68), 7.62-7.54 (1H, m, N*H*-amide), 7.42-7.38 (2H, m, C*H*-61, 66), 7.30-7.24 (4H, m, C*H*-26,30,60,67), 7.14-7.09 (4H, m, C*H*-44, 45,47,48), 7.03 (2H, d, *J* 7.9 Hz, C*H*-27,29), 7.00-6.98 (1H, m, C*H*-46), 6.75 (1H, m, N*H*-guanidine), 6.41 (2H, m, N*H*-guanidine, N*H*-sulfonamide), 4.63-4.48 (3H, m, C*H*-4, 23,41), 4.35-4.17 (4H, m, C*H*-50,57, CH_2-56), 3.80-3.17 (2H, m, CH_2-2), 3.09-3.01 (4H, m, CH_2-7, CH_2-24), 2.94 (2H, s, CH_2-18), 2.77-2.72 (1H, m, CH_AH$_B$-42), 2.58-2.31 (8H, m, CH_3-11, 21, CH$_A$$H_B$-42, C$H_AH_B$-51), 2.17 (1H, dd, *J* 15.4, 10.7 Hz, CH$_A$$H_B$-51), 1.99 (3H, s, C$H_3$-13), 1.73-1.67 (1H, m, CH_AH$_B$-5) and 1.57-1.33 (18H, m, CH$_A$$H_B$-5, C$H_2$-6, C$H_3$-16,17, 3 × C$H_3$-54); **^{13}C NMR** (125 MHz, d_6-DMSO) δ 175.4 (quat., C-32), 172.0, 171.6, 171.1, 171.0, 170.8, 169.6 (quat. × 6, C-1,3,22,40,49,52), 157.9 (quat., C-14), 157.9 (quat., C-31), 156.6 (quat., C-8), 156.2 (quat., C-55), 150.0 (quat., C-28), 144.3, 144.1 (quat., C-58,69), 141.1 (quat. × 2, C-59,68), 137.8 (quat., C-9), 134.7 (quat., C-19), 134.4 (quat., C-34), 134.1 (quat., C-25), 134.0 (*C*H, C-37), 131.9 (quat., C-12), 130.7 (*C*H × 2, C-35,39), 130.5 (*C*H × 2, C-27, 29), 130.4 (*C*H × 2, C-45,47), 129.7 (*C*H × 2, C-44,48), 128.3 (quat., C-43), 128.1 (*C*H × 2, C-61,66), 127.5 (*C*H × 2, C-60,67), 126.6 (*C*H, C-46), 125.7 (*C*H × 2, C-59,68), 124.8 (quat., C-10), 122.9 (*C*H × 2, C-35,39), 121.7 (*C*H × 2, C-26,30), 120.5 (*C*H × 2, C-52,65), 116.7 (quat., C-20), 105.2 (*C*H, C-33), 86.7 (quat., C-15), 80.5 (quat., C-53), 66.2 (*C*H_2, C-56), 54.3 (*C*H, C-4), 54.1 (*C*H, C-23), 52.6 (*C*H, C-41), 51.9 (*C*H, C-50), 47.0 (*C*H, C-57), 42.9 (*C*H_2, C-18), 41.3 (*C*H_2, C-2), 40.6 (*C*H_2, C-7), 38.4 (*C*H_2, C-42), 38.1 (*C*H_2, C-51), 37.4 (*C*H_2, C-24), 30.0 (*C*H_2, C-5), 28.8 (*C*H_2 × 2, CH_3-16,17), 28.1 (*C*H_3 × 3, C-54), 25.8 (*C*H_2, C-6), 19.4 (*C*H_3, C-13), 18.1 (*C*H_3, C-11) and 12.7 (*C*H_3, C-21); **HRMS** *m/z* (ES^+) calcd. for $C_{54}H_{56}N_9O_{12}S$ $[M+Na]^+$ requires 1396.5319, found 1396.5321.

1,5-Di-*tert*-butyl (2*S*)-2-((((2*S*)-6-(((benzyloxy)carbonyl)amino)-1-(*tert*-butoxy)-1-oxohexan-2-yl)carbamoyl)amino)pentanedioate, 382[257]



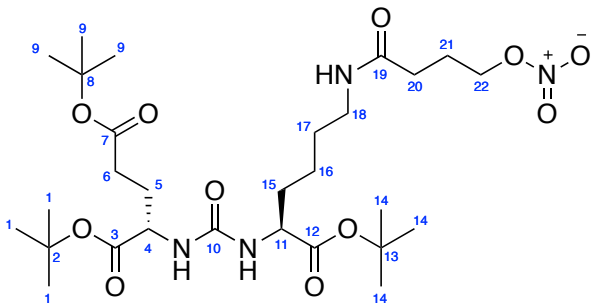
A solution of *N*-ε-Cbz-lysine *tert*-butyl ester hydrochloride **384** (0.91 g, 2.70 mmol) and diisopropylethylamine (0.79 g, 1.05 mL, 6.00 mmol) in dry dichloromethane (5 mL) was added *via* syringe pump over 1 hour to a solution of triphosgene (0.29 g, 1.00 mmol) in dry dichloromethane (5 mL) at 0 °C. The solution was stirred for a further hour at 0 C. After this time a solution of glutamic acid di-*tert*-butyl ester hydrochloride **383** (0.80 g, 2.70 mmol) and diisopropylethylamine (0.79 g, 1.05 mL, 6.00 mmol) in dry dichloromethane (5 mL) was added in one portion. The solution was allowed to warm to room temperature and stirred for a further two h. The solvent was removed under reduced pressure and the residue diluted with ethyl acetate (50 mL), washed with aq. HCl (2 N, 2 × 50 mL), brine (100 mL), dried over Na_2SO_4. The solvent was removed under reduced pressure and the residue uptaken in dichloromethane and adsorbed onto silica gel. Silica gel chromatography, eluting with ethyl acetate and hexane (10:90 to 40:60) provided 1,5-di-*tert*-butyl (2*S*)-2-((((2*S*)-6-(((benzyloxy)carbonyl)amino)-1-(*tert*-butoxy)-1-oxohexan-2-yl)carbamoyl)amino)pentanedioate **382** (1.31 g, 2.11 mmol) as a colourless oil: $\boldsymbol{R_f}$ 0.69 (50:50, EtOAc:PE, weakly UV active/$KMnO_4$); $[\boldsymbol{\alpha}]_{\mathbf{D}}^{\mathbf{20}}$ +8.4, (*c* = 1, $CHCl_3$); **^{1}H NMR** (500 MHz; $CDCl_3$) δ 7.34-7.25 (5H, m, C*H*-22,23,24,25,26), 5.56-5.55 (2H, m, N*H*-Cbz, N*H*-Glu-urea), 5.47 (1H, d, *J* 5.6, N*H*-Lys-urea), 5.13-5.03 (2H, m, CH_2-20), 4.36 (1H, dd, *J* 12.2, 7.4 Hz, C*H*-4), 4.30 (1H, dd, *J* 11.2, 6.3 Hz, C*H*-11), 3.14 (2H, dt, *J* 7.2, 6.3 Hz, CH_2-18), 2.27-2.21 (2H, m, CH_2-6), 2.04-1.98 (1H, m, CH_AH$_B$-5), 1.81-1.74 (1H, m, CH$_A$*H*$_B$-5), 1.73-1.66 (1H, m, C*H*$_A$H$_B$-15), 1.60-1.52 (1H, m, C*H*$_A$H$_B$-15), 1.45-1.37 (29H, CH_2-17, 3 × CH_3-1, 3 × CH_3-9, 3 × CH_3-14) and 1.33-1.24 (2H, m, CH_2-16); **^{13}C NMR** (125 MHz; $CDCl_3$) δ 173.0 (quat., C-3), 172.7 (quat., C-7), 172.3 (quat., C-12), 156.7 (quat., C-19), 157.2 (quat., C-10), 136.8 (quat., C-21), 128.0 (*C*H × 2, C-22,26), 127.9 (*C*H, C-21), 123.4 (*C*H × 2, C-23,25), 82.0 (quat., C-2), 82.0 (quat., C-13), 80.4 (quat., C-8), 66.4 (*C*H_2, C-20), 53.3 (*C*H, C-11), 52.8 (*C*H, C-4), 40.7 (*C*H_2, C-18), 32.6 (*C*H_2, C-15), 31.6 (*C*H_2, C-6), 29.3 (*C*H_2, C-16), 28.3 (*C*H_2, C-5), 28.0 (*C*H_3 × 6, C-1, 9), 28.0 (*C*H_3 × 3, C-14), 22.4 (*C*H_2, C-17); ***m/z*** (ES$^+$) 644 ([M+Na]$^+$, 100%). The data were in agreement with the literature values.[257]

1,5-Di-*tert*-butyl (2*S*)-2-((((2*S*)-6-amino-1-(*tert*-butoxy)-1-oxohexan-2-yl)carbamoyl)-amino)pentanedioate, 385[257]



A solution of Cbz amine **382** (500 mg, 0.80 mmol) in methanol (27 mL, 0.03 M) was hydrogenated at room temperature in flow (H-Cube, 1 mL min^{-1}) over a 10% Pd/C carbon cartridge. The solvent was evaporated under reduced pressure to yield **385** (406 mg, 0.80 mmol, quant.) as a waxy solid which was used immediately: R_f = 0.00 (50:50, EtOAc:PE, $KMnO_4$); **^{1}H NMR** (500 MHz; $CDCl_3$) δ 5.35-5.32 (2H, m, N*H*-urea × 2), 4.34-4.29 (2H, m, C*H*-4,11), 2.66 (2H, t, *J* 6.5 Hz, CH_2-18), 2.34-2.21 (2H, m, CH_2-6), 2.07-2.00 (1H, m, CH_AH$_B$-5), 1.85-1.70 (2H, m, CH$_A$$H_B$-5, C$H_AH_B$-15), 1.62-1.54 (1H, m, CH$_A$$H_B$-15), 1.56-1.30 (31H, 3 × CH_3-1, 3 × CH_3-9, 3 × CH_3-14, CH_2-16, CH_2-17); **^{13}C NMR** (125 MHz; $CDCl_3$) δ 172.7, 172.5, 172.3 (quat., × 3, C-3,7,18), 156.9 (quat., C-10), 82.0, 81.6, 80.5 (quat. × 3, C-2,8,13), 53.1 (*C*H, C-4), 53.0 (*C*H$_2$, C-11), 41.8 (*C*H$_2$, C-18), 33.1 (*C*H$_2$, C-6), 32.9 (*C*H$_2$, C-15), 31.6 (*C*H$_2$, C-17), 28.4 (*C*H$_2$, C-5), 28.1, 28.0, 28.0 (*C*H$_3$ × 9, C-1,9,14), 22.4 (*C*H$_2$, C-16); ***m/z*** (ES$^+$) 510 ([M+Na]$^+$, 100%). The data were in agreement with the literature values.[257]

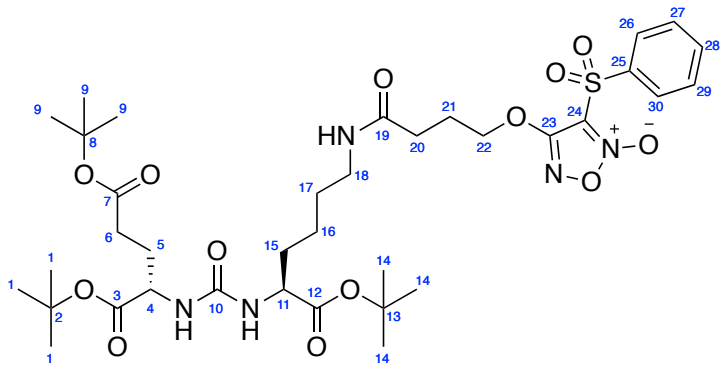
1, 5-Di-*tert*-butyl (2*S*)-2-((((2*S*)-1-(*tert*-butoxy)-6-(4-(nitrooxy)butanamido)-1-oxohexan-2-yl)carbamoyl)amino)pentanedioate, 386



Following general procedure B with HATU (510 mg, 1.34 mmol), 4-(nitrooxy)butanoic acid **258** (100 mg, 0.67 mmol), amine **385** (218 mg, 0.45 mmol) and diisopropylethylamine

(distilled, 231 mg, 310 µL, 1.79 mmol). Purification by silica gel chromatography, eluting with dichloromethane and methanol (98:2) provided *1, 5-di-tert-butyl (2S)-2-((((2S)-1-(tert-butoxy)-6-(4-(nitrooxy)butanamido)-1-oxohexan-2-yl)carbamoyl)amino)pentanedioate* **386** (194 mg, 0.31 mmol, 70%) as an amber gum: R_f 0.58 (90:10, CH_2Cl_2:MeOH, UV/cerium phosphomolybdate); $[\alpha]_D^{20}$ +9.2, (*c* = 0.1, $CHCl_3$); ν_{max} (thin film)/cm^{-1} 1730, 1635, 1561, 1458, 1368, 1277, 1257, 1153, 1036, 846, 748, 665; **^{1}H NMR** (500 MHz; $CDCl_3$) δ 7.04 (1H, t, *J* 5.4, N*H*-amide), 5.84 (1H, d, *J* 7.6, N*H*-urea-Lys), 5.50 (1H, d, *J* 6.2, N*H*-urea-Glu), 4.50 (2H, t, *J* 7.6 Hz, CH_2-22), 4.30 (1H, dt, *J* 8.6, 5.0 Hz, C*H*-11), 4.17-4.11 (1H, m, C*H*-4), 3.33-3.25 (1H, m, CH_AH$_B$-18), 3.16-3.09 (1H, m, CH_AH$_B$-18), 2.37-2.29 (4H, m, CH_2-6, 20), 2.08-2.02 (3H, m, CH_AH$_B$-5, CH_2-21,), 1.85-1.77 (1H, m, CH$_A$$H_B$-5), 1.74-1.67 (1H, m, C$H_AH_B$-15), 1.55-1.49 (1H, m, CH_AH$_B$-15); 1.48-1.36 (29H, m, 3 × CH_3-1, 3 × CH_3-9, 3 × CH_3-14, CH_2-17), 1.31-1.24 (2H, m, CH_2-16); **^{13}C NMR** (125 MHz; $CDCl_3$) δ 172.4, 172.2, 171.8 (quat. × 3, C-3,7,12), 157.5 (quat., C-10), 82.6, 81.5, 80.7 (quat. × 3, C-2,8,13), 72.8 (*C*H$_2$, C-22), 53.7 (*C*H, C-4), 53.1 (*C*H, C-11), 39.0 (*C*H$_2$, C-18), 32.4 (*C*H$_2$ × 2, C-6, C-20), 31.6 (*C*H$_2$, C-17), 28.8 (*C*H$_2$, C-5), 28.0 (*C*H$_3$ × 9, C-1,9,14), 27.9 (*C*H$_2$, C-15), 23.2 (*C*H$_2$, C-21) and 22.9 (*C*H$_2$, C-16); ***m/z*** (ES$^+$) 556 ([M−ONO$_3$]$^+$, 100%), 641 ([M+Na]$^+$, 50%); **HRMS** *m/z* (ES$^+$) calcd. for $C_{28}H_{50}N_4NaO_{11}$ [M+Na]$^+$ requires 641.3374, found 641.3354.

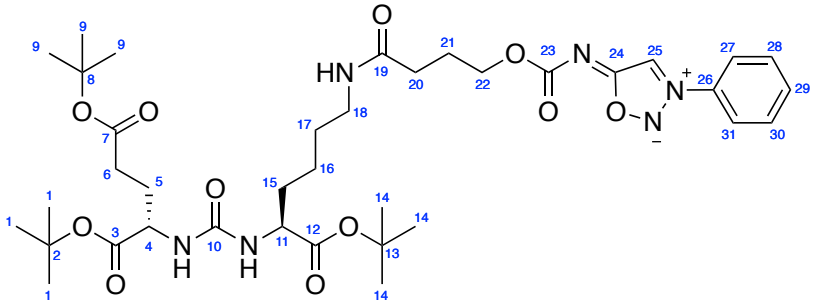
1, 5-Di-*tert*-butyl (2*S*)-2-((((2*S*)-1-(*tert*-butoxy)-6-(4-((3-phenylsulfonyl)-1,2,5-oxadiazole 2-oxide)oxy)butanamido)-1-oxohexan-2-yl)carbamoyl)amino) pentanedioate, 387



Following general procedure B with HATU (468 mg, 1.23 mmol), 4-(3-carboxypropoxy)-3-(phenylsulfonyl)-1,2,5-oxadiazole 2-oxide **261** (148 mg, 0.45 mmol), amine **385** (200 mg, 0.41 mmol) and diisopropylethylamine (distilled, 212 mg, 281 µL, 1.64 mmol). Purification by silica gel chromatography, eluting with dichloromethane and methanol (98:2) provided *1, 5-di-tert-butyl (2S)-2-((((2S)-1-(tert-butoxy)-6-(4-((3-phenylsulfonyl)*-1,2,*5-oxadiazole 2-oxide)oxy)butanamido)-1-oxohexan-2-yl)carbamoyl)amino) pentanedioate* **387** (229 mg, 0.29

mmol, 70%) as a white solid: $\boldsymbol{R_f}$ 0.60 (90:10, CH_2Cl_2:MeOH, UV/cerium phosphomolybdate); **m.p.** 94-97 °C; $[\alpha]_D^{20}$ +13.6, (*c* = 0.1, $CHCl_3$); $\boldsymbol{\nu_{max}}$ (thin film)/cm^{-1} 1733, 1633, 1552, 1451, 1367, 1257, 1157, 1086, 1021, 958, 899, 848, 748; **^{1}H NMR** (500 MHz; $CDCl_3$) δ 8.04 (2H, d, *J* 7.6 Hz, C*H*-26,30), 7.75 (1H, t, *J* 7.3 Hz, C*H*-28), 7.62 (2H, t, *J* 7.8 Hz, C*H*-27,29), 6.61 (1H, t, *J* 5.4, N*H*-amide), 5.57 (1H, d, *J* 8.0, N*H*-urea-Lys), 5.44 (1H, d, *J* 7.7, N*H*-urea-Glu), 4.48 (2H, t, *J* 6.2 Hz, CH_2-22), 4.31 (1H, dt, *J* 8.0, 5.0 Hz, C*H*-11), 4.24 (1H, dt, *J* 7.7, 4.4 Hz, C*H*-4), 3.33-3.37 (1H, m, CH_AH$_B$-18), 3.20-3.14 (1H, m, CH$_A$$H_B$-18), 2.41 (2H, t, *J* 6.9 Hz, CH_2-20), 2.34-2.26 (2H, m, CH_2-6), 2.21 (2H, tt, *J* 6.9, 6.2 Hz, CH_2-21), 2.09-2.01 (1H, m, CH_AH$_B$-5), 1.86-1.79 (1H, m, CH$_A$$H_B$-5), 1.78-1.71 (1H, m, C$H_AH_B$-15), 1.61-1.30 (32H, m, 3 × CH_3-1, 3 × CH_3-9, 3 × CH_3-14, CH$_A$$H_B$-15, C$H_2$-16,17); **^{13}C NMR** (125 MHz; $CDCl_3$) δ 173.1, 172.4, 172.3, 171.9 (quat. × 4, C-3,7,12,19),158.9 (quat., C-24), 157.3 (quat., C-10), 137.9 (quat., C-25), 135.7 (*C*H, C-28), 129.7 (*C*H × 2, C*H*-26,30), 128.5 (*C*H × 2, C*H*-27, 29), 110.5 (quat., C-23), 82.3, 81.6, 80.6 (quat. × 3, C-2,8,13), 70.8 (*C*H_3, C-22), 53.4 (*C*H, C-4), 53.0 (*C*H, C-11), 39.0 (*C*H_2, C-18), 32.4 (*C*H_2, C-6), 31.9 (*C*H_2, C-20), 31.5 (*C*H_2, C-17) 28.8 (*C*H_2, C-5), 28.1 (*C*H_2, C-15), 28.0 (3 × CH3 × 3, C-1,9,14), 24.5 (*C*H_2, C-21) and 22.6 (*C*H_2, C-16); ***m/z*** 820 ([M+Na]$^+$, 100%); **HRMS** *m/z* (ES$^+$) calcd. for $C_{36}H_{55}N_5NaO_{13}$ [M+Na]$^+$ requires 820.3415, found 820.3392.

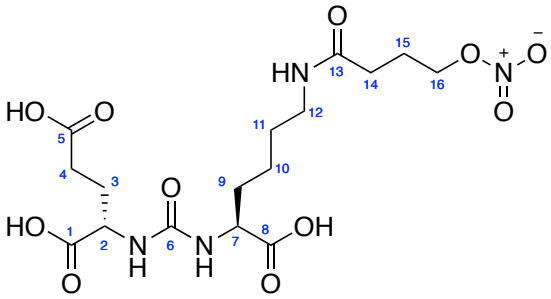
N-(((3-(((5*S*)-5-((((2*S*)-1,5-*Bis*(*tert*-butoxy)-1,4-dioxopentan-2-yl)carbamoyl)amino)-6-(*tert*-butoxy)-6-oxohexyl)carbamoyl)propoxy)carbonyl)-3-phenylsydnonimine, 388



Following general procedure B with HATU (304 mg, 0.80 mmol), *N*-((3-carboxypropxy)carbonyl)-3-phenylsydnonimine **266** (96 mg, 0.29 mmol), amine **385** (130 mg, 0.27 mmol) and diisopropylethylamine (distilled, 137 mg, 190 μL, 1.08 mmol). Purification by silica gel chromatography, eluting with dichloromethane and methanol (98:2) provided *N-(((3-(((5S)-5-((((2S)-*1,5*-bis(tert-butoxy)-*1,4*-dioxopentan-2-yl)carbamoyl)amino)-6-(tert-butoxy)-6-oxohexyl)carbamoyl)propoxy)carbonyl)-3-phenylsydnonimine* **388** (148 mg, 0.19 mmol, 66%) as an amber solid: $\boldsymbol{R_f}$ 0.69 (90:10, CH_2Cl_2:MeOH,, UV/cerium phosphomolybdate); **m.p.** 44-46

°C; $[\alpha]_D^{20}$ +13.6, (*c* = 0.1, $CHCl_3$); ν_{max} (thin film)/cm^{-1} 1735, 1649, 1550, 1428, 1370, 1265, 1150, 1099, 900; **^{1}H NMR** (500 MHz; $CDCl_3$) δ 8.42 (1H, s, C*H*-25), 7.93 (2H, d, *J* 8.5 Hz, C*H*-27,31), 7.70 (1H, tt, *J* 7.5, 1.2 Hz, C*H*-29), 7.64 (2H, t, *J* 8.5 Hz, C*H*-28,30), 6.71 (1H, t, *J* 5.8, N*H*-amide), 6.10 (1H, d, *J* 9.1, N*H*-urea-Lys), 5.92 (1H, d, *J* 8.8, N*H*-urea-Glu), 4.42 (1H, ddd, *J* 13.1, 8.8, 4.3 Hz, C*H*-11), 4.30-4.20 (2H, m, C*H*-4, CH_AH_B-22), 4.15-4.09 (1H, m, CH_AH_B-22), 3.54-3.47 (1H, m, CH_AH_B-18), 2.98-2.90 (1H, m, CH_AH_B-18), 2.36-2.27 (4H, m, CH_2-6, CH_2-20), 2.18-1.98 (3H, m, CH_AH_B-15, CH_2-21), 1.86-1.76 (2H, m, CH_AH_B-15, CH_AH_B-17), 1.47-1.29 (32H, 3 × CH_3-1, CH_2-5, 3 × CH_3-9, 3 × CH_3-14, CH_2-16, CH_AH_B-17); **^{13}C NMR** (125 MHz; $CDCl_3$) δ 175.1 (quat., C-24), 173.3, 172.9, 172.7, 172.2 (quat., × 5, C-3,7,12,19), 161.7 (quat., C-23), 157.8 (quat., C-10), 133.8 (quat., C-26), 133.1 (*C*H, C-29), 130.5 (*C*H × 2, C-19,23), 121.8 (*C*H × 2, C-27,31), 103.7 (*C*H, C-25) 64.8 (CH_2, C-22), 53.3 (*C*H, C-4), 52.8 (*C*H, C-11), 38.7 (CH_2, C-18), 32.7 (CH_2, C-20), 32.5 (CH_2, C-6), 31.9 (CH_2, C-17), 28.5 (CH_2, C-5), 28.0, 28.0, 28.0 (CH_3 × 9, C-1,9,14), 27.7 (CH_2, C-15), 21.7 (CH_2, C-16) and 25.0 (CH_2, C-21),; ***m/z*** 783 ($[M+Na]^+$, 100%); **HRMS** *m/z* (ES^+) calcd. for $C_{37}H_{56}N_6NaO_{11}$ $[M+Na]^+$ requires 783.3899, found 783.3900.

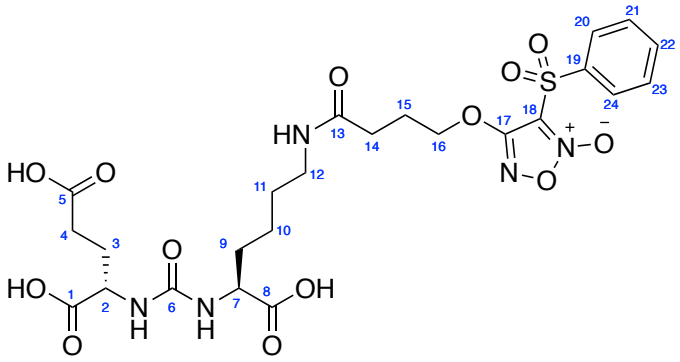
(2*S*)-2-((((2*S*)-1-Carboxy-5-(4-(nitrooxy)butanamido)pentyl)carbamoyl)amino) pentanedioic acid, 389



Trifluoroacetic acid (1 mL, 13.1 mmol) was added to a solution of amide **386** (100 mg, 0.16 mmol) in dichloromethane (4 mL) cooled in an ice-bath. The solution was stirred for 18 h. The solvent was removed under reduced pressure and the residue evaporated from toluene, methanol, and dichloromethane. The residue was triturated with cold acetonitrile to furnish *(2S)-2-((((2S)-1-carboxy-5-(4-(nitrooxy)butanamido)pentyl)carbamoyl)amino) pentanedioic acid* **389** (66 mg, 0.15 mmol, 90%) as a colourless gum: R_f = 0.00 (CH_2Cl_2, cerium phosphomolybdate); ν_{max} (KBr disc)/cm^{-1} 2930, 2909, 1725, 1600, 1478, 1300, 1270, 1257, 1140, **^{1}H NMR** (500 MHz, d_6-DMSO) δ 12.47 (3H, s, *br*, 3 × COOH), 7.87 (1H, t, *J* 4.9 Hz, N*H*-amide), 6.36-6.30 (2H, m, 2 × N*H*-urea), 4.51 (2H, t, *J* 6.0 Hz, CH_2-16), 4.12-4.04 (2H, m, C*H*-2, 7), 3.01 (2H, dd, *J* 12.8, 6.6 Hz, CH_2-12), 2.32-2.14 (4H, m, CH_2-4, CH_2-14), 1.96-1.84 (3H, m, CH_AH_B-3, CH_2-15), 1.74-1.48 (3H, m, CH_AH_B-3, CH_2-9) and 1.41-1.22 (4H, m, CH_2-

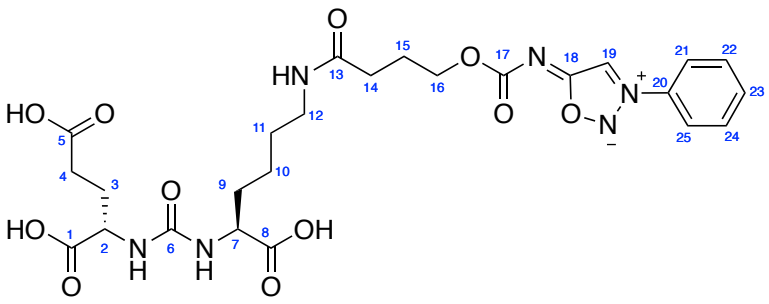
11, CH_2-10); **^{13}C NMR** (125 MHz, d_6-DMSO) δ 175.0, 174.6, 174.2, 171.2 (quat. × 4, C-1,5,8,13), 157.7 (quat., C-6), 73.8 (CH_2, C-16), 52.6, 52.1 (CH × 2, C-2,7), 38.8 (CH_2, C-12), 32.3 (CH_2, C-9), 31.7 (CH_2, C-4), 30.4 (CH_2, C-14), 29.3 (CH_2, C-11), 28.0 (CH_2, C-3), 23.0 (CH_2, C-10) and 22.7 (CH_2, C-15); ***m/z*** (ES$^-$) 449 ([M-H]$^-$, 100%); **HRMS** *m/z* (ES$^-$) calcd. for $C_{16}H_{25}N_4O_{11}$ [M-H]$^-$ requires 449.1525, found 449.1530.

(2*S*)-2-((((2*S*)-1-Carboxy-5-(4-((3-phenylsulfonyl)-1,2,5-oxadiazole 2-oxide)oxy)butan-amido)pentyl)carbamoyl)amino) pentanedioic acid, 390



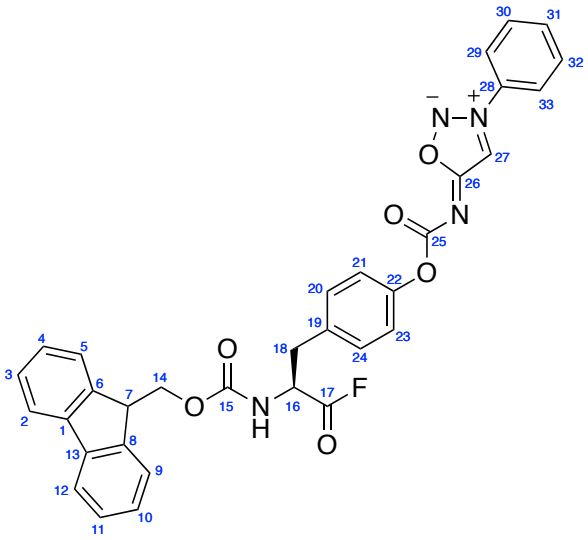
Trifluoroacetic acid (1 mL, 13.1 mmol) was added to a solution of amide **387** (100 mg, 0.13 mmol) in dichloromethane (4 mL) cooled in an ice-bath. The solution was stirred for 18 h. The solvent was removed under reduced pressure and the residue evaporated from toluene, methanol, and dichloromethane. The residue was triturated with cold acetonitrile to furnish *(2S)-2-((((2S)-1-carboxy-5-(4-((3-phenylsulfonyl)-1,2,5-oxadiazole 2-oxide)oxy)butanamido)-pentyl)carbamoyl)amino) pentanedioic acid* **390** (73 mg, 0.12 mmol, 92%) as a white solid: ***R$_f$*** = 0.00 (CH_2Cl_2, UV/cerium phosphomolybdate); **m.p.** 77 °C (decomp.); **ν_{max}** (KBr disc)/cm^{-1} 2944, 2908, 1730, 1622, 1500, 1388, 1257, 1158, 1091, 1022, 900; **^{1}H NMR** (500 MHz, d_6-DMSO) δ 12.45 (3H, s, *br*, 3 × COO*H*), 8.03 (2H, d, *J* 7.7 Hz, C*H*-20,24), 7.92-7.87 (2H, m, C*H*-22, N*H*-amide), 7.75 (2H, t, *J* 7.7 Hz, C*H*-21,23), 6.37-6.27 (2H, m, 2 × N*H*-urea), 4.40 (2H, t, *J* 6.2 Hz, CH_2-16), 4.12-4.02 (2H, m, C*H*-2, 7), 3.03 (2H, dd, *J* 13.3, 6.8 Hz, CH_2-12), 2.30-2.19 (4H, m, CH_2-4, CH_2-14), 2.01-1.87 (3H, m, CH_AH$_B$-3, CH_2-15), 1.75-1.60 (3H, m, CH$_A$$H_B$-3, C$H_2$-9), 1.43-1.38 (2H, m, C*H*-11) and 1.32-1.25 (2H, m, CH_2-10); **^{13}C NMR** (125 MHz, d_6-DMSO) δ 175.0, 174.3, 174.2, 171.4 (quat. × 4, C-1, 5, 8, 13), 159.3 (quat., C-17), 157.7 (quat., C-6), 137.6 (quat., C-19), 136.6 (*C*H, C-22), 130.5 (*C*H × 2, C-21,23), 128.8 (*C*H × 2, C-21,23), 110.0 (quat., C-18), 79.6 (CH_2, C-16), 52.7, 52.0 (*C*H × 2, C-2, 7), 38.8 (CH_2, C-12), 32.3 (CH_2, C-9), 31.5 (CH_2, C-4), 30.4 (CH_2, C-14), 29.3 (CH_2, C-11), 28.0 (CH_2, C-3), 24.7 (CH_2, C-15) and 23.1 (CH_2, C-10); ***m/z*** (ES$^+$) 628 ([M-H]$^-$, 100%); **HRMS** *m/z* (ES$^+$) calcd. for $C_{24}H_{30}N_5O_{13}S$ [M-H]$^-$ requires 628.1566, found 628.1576.

5-(((3-((((5*S*)-5-Carboxy-5-((((1*S*)-1,3-dicarboxypropyl)carbamoyl)amino)-pentyl)-carbamoyl)propoxy)carbonyl)-3-phenylsydnonimine, 391



Trifluoroacetic acid (1 mL, 13.1 mmol) was added to a solution of amide **388** (100 mg, 0.13 mmol) in dichloromethane (4 mL) cooled in an ice-bath. The solution was stirred for 18 h. The solvent was removed under reduced pressure and the residue evaporated from toluene, methanol, and dichloromethane. The residue was triturated with cold acetonitrile to furnish *5-(((3-((((5S)-5-carboxy-5-((((1S)*-1,3-*dicarboxypropyl)carbamoyl)amino)pentyl)carbamoyl)-propoxy)carbonyl)-3-phenylsydnonimine* **391** (62 mg, 0.11 mmol, 80%) as a off-white solid: $\boldsymbol{R_f}$ = 0.00 (CH_2Cl_2, UV/cerium phosphomolybdate); **m.p.** 82 °C (decomp.); $\boldsymbol{\nu_{max}}$ (KBr disc)/cm^{-1} 1724, 1701, 1615, 1462, 1395, 1249, 1160, 1090, 1025, 898; **^{1}H NMR** (500 MHz, d_6-DMSO) δ 12.35 (3H, s, *br*, 3 × CO_2H), 8.56 (1H, s, C*H*-19), 8.07 (2H, d, *J* 7.5 Hz, C*H*-21, 25), 7.85 (1H, t, *J* 5.3 Hz, N*H*-amide), 7.78 (1H, tt, *J* 7.3, 1.2 Hz, C*H*-23), 7.73 (2H, t, *J* 7.3 Hz, C*H*-22,24), 6.34-6.25 (2H, m, 2 × N*H*-urea), 4.11-3.98 (4H, m, C*H*-2, 7, CH_2-16), 3.01 (2H, dd, *J* 12.4, 6.3 Hz, CH_2-12), 2.30-2.12 (4H, m, CH_2-4,14), 1.94-1.87 (1H, m, CH_AH_B-3), 1.81 (2H, dd, *J* 7.3, 7.3 Hz, CH_2-15), 1.76-1.68 (1H, m, CH_AH_B-3), 1.67-1.60 (1H, m, CH_AH_B-9), 1.54-1.47 (1H, m, CH_AH_B-9), 1.41-1.34 (2H, m, CH_2-11) and 1.31-1.25 (2H, m, CH_2-10); **^{13}C NMR** (125 MHz, d_6-DMSO) δ) **^{13}C NMR** (125 MHz, d_6-DMSO) δ 175.4 (quat., C-18), 175.0, 174.7, 173.3, 171.8 (quat. × 4, C-1,5,8,13), 160.8 (quat., C-17), 157.8 (quat., C-6), 134.2 (quat., C-20), 133.5 (CH, 133.5), 130.7 (*C*H × 2, C-22,24), 122.9 (*C*H × 2, C-21,25), 104.5 (quat., C-19), 64.5 (CH_2, C-16), 52.9, 52.5 (*C*H × 2, C-2,7), 38.9 (CH_2, C-12), 32.3 (CH_2, C-9), 31.8 (CH_2, C-4), 30.8 (CH_2, C-14), 29.4 (CH_2, C-11), 29.0 (CH_2, C-3), 25.3 (CH_2, C-15) and 23.2 (CH_2, C-10); ***m/z*** (ES^+) 591 ($[M-H]^-$, 100%); **HRMS** *m/z* (ES^+) calcd. for $C_{25}H_{31}N_6O_{11}$ $[M-H]^-$ requires 591.2056, found 591.2060.

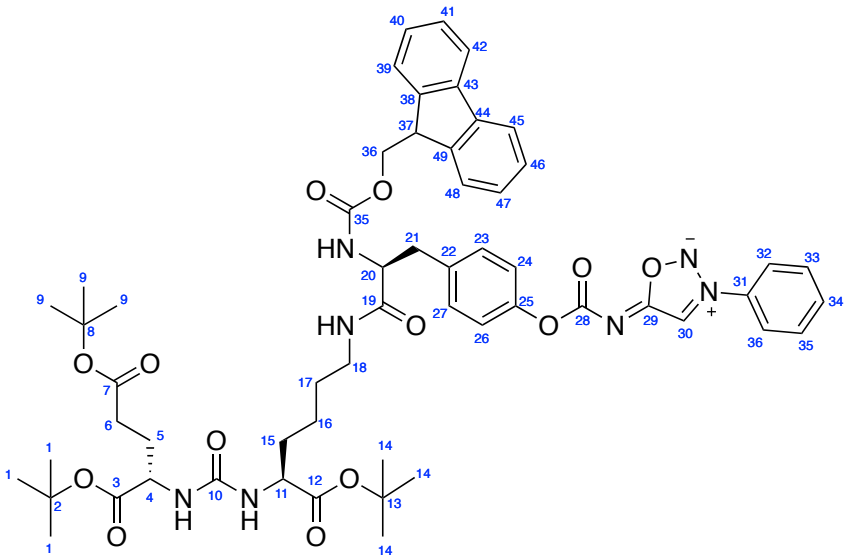
_N_-((4-(((2_S_)-2-((((9H-Fluoren-9-ylmethoxy)carbonyl)amino)-3-fluoro-3-oxopropyl)-phenoxycarbonyl)-3-phenylsydnonimine, 392



Cyanuric fluoride (114 mg, 73 µL, 0.85 mmol) was added to a solution of (*S*)-*N*-(((4-(2-(((9*H*-fluoren-9-yl)methoxy)carbonyl)amino)-2-carboxyethyl)phenoxy)carbonyl)-3-phenylsydnonimine **321** (250 mg, 0.42 mmol) in dry dichloromethane (8 mL). To this was added pyridine (35 µL, 0.42 mmol) and the solution was stirred for 3 h. The resulting suspension was diluted with dichloromethane (10 mL) and washed with ice-cold water (20 mL × 2), dried over $MgSO_4$. The mixture was filtered and the solvent removed under reduced pressure to give *N-((4-(((2S)-2-((((9H-fluoren-9-ylmethoxy)carbonyl)amino)-3-fluoro-3-oxopropyl)phenoxycarbonyl)-3-phenylsydnonimine* **392** (247 mg, 0.41 mmol, quant.) as an orange solid: **m.p.** 109-113 °C, $[\alpha]_D^{20}$ +31.6 (*c* = 1.0, $CHCl_3$); ν_{max} (KBr disc)/cm^{-1} 1840, 1740, 1680, 1586, 1510, 1469, 1450, 1350, 1215, 1190; **^{1}H NMR** (500 MHz, d_6-DMSO) δ 8.52 (1H, s, C*H*-27), 8.17 (1H, d, *J* 7.3, N*H*-Fmoc), 8.01 (2H, d, *J* 7.9 Hz, C*H*-2,12), 7.85 (2H, d, *J* 7.5 Hz, C*H*-29,33), 7.76 (1H, t, *J* 7.4 Hz, C*H*-31), 7.70 (2H, t, *J* 8.0 Hz, C*H*-30,32), 7.61 (2H, t, *J* 7.0 Hz, C*H*-5,9), 7.39 (2H, d, *J* 7.5 Hz, C*H*-3,11), 7.30 (2H, *J* 6.2, C*H*-4,10), 7.23 (2H, d, *J* 8.5 Hz, C*H*-20,24), 7.02 (2H, d, *J* 8.5 Hz, C*H*-21,23), 4.55-4.50 (1H, m, C*H*-16), 4.38 (1H, dd, *J* 10.6, 6.9 Hz, CH_AH_B-14), 4.30 (1H, dd, *J* 10.6, 6.6 Hz, CH_AH_B-14), 4.18 (1H, t, *J* 6.7 Hz, C*H*-7), 3.12 (1H, dd, *J* 13.8, 5.0 Hz CH_AH_B-18) and 2.99 (1H, dd, *J* 13.8, 10.2 Hz, CH_AH_B-18; **^{13}C NMR** (125 MHz, d_6-DMSO) δ 175.4 (quat., C-26), 174.6 (quat., *J* 192.7 Hz C-17), 159.3 (quat., C-25), 156.6 (quat., C-15), 150.9 (quat., C-22), 147.1 (quat., C-6,8), 141.1 (quat., C-1,13), 133.9 (quat., C-19), 134.8 (quat., C-28), 133.6 (*C*H, C-31), 130.9 (*C*H × 2, C-30,32), 130.3 (*C*H × 2, C-20,24) 128.2 (quat., C-3,11), 127.6 (quat., C-4,10), 125.6 (quat., C-5,9), 122.8 (*C*H × 2, C-29,33), 122.0 (*C*H × 2, C-21,23), 120.5 (quat., C-2,12), 105.2 (*C*H, C-27), 66.1 (CH_2, C-14),

56.0 (*CH*, C-16), 47.0 (*CH*, C-7) and 36.6 (CH_2, C-18); **^{19}F NMR** (376 MHz, d_6-DMSO) δ +30.5 (CO*F*); ***m/z*** (ES^+) 593 ($[M+H]^+$, 100%); **HRMS** *m/z* (ES^+) calcd. for $C_{33}H_{26}N_4O_6$ $[M+H]^+$ requires 593.1831, found 593.1839.

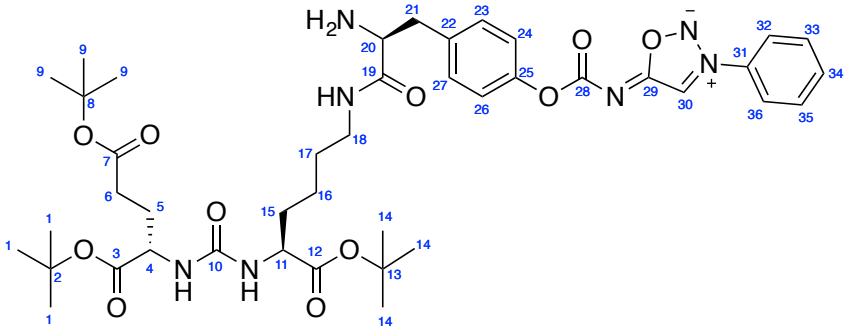
N-((4-(((2*S*)-2-((((5*S*)-5-((((2*S*)-1,5-*Bis*(*tert*-butoxy)-1,5-dioxopentan-1-yl)carbamoyl)-amino-6-(*tert*-butoxy)-6-oxohexyl)carbamoyl)-2-((((9H-fluoren-9-ylmethoxy)-carbonyl)amino)ethyl)phenoxycarbonyl)-3-phenylsydnonimine, 393



Acyl fluoride **392** (458 mg, 0.77 mmol in dry CH_2Cl_2, 10 mL) was added dropwise to a solution of amine **385** (344 mg, 0.71 mmol) and diispropylethylamine (distilled, 200 mg, 266 µL, 1.55 mmol) in dry dichloromethane (20 mL) cooled to -20 °C and stirred for 1 hour. The solution as diluted with dichloromethane (20 mL) and washed with saturated sodium bicarbonate (30 mL), brine (30 mL). The organic layer was separated and dried over Na_2SO_4, filtered and the solvent removed under reduced pressure. The resultant residue was uptaken in dichloromethane and adsorbed onto silica gel. Purification by silica gel chromatography eluting with dichloromethane and methanol (98:2) provided *N-((4-(((2S)-2-((((5S)-5-((((2S)*-1,5-*bis(tert-butoxy)*-1,5-*dioxopentan-1-yl)carbamoyl)amino-6-(tert-butoxy)-6-oxohexyl)carbamoyl)-2-((((9H-fluoren-9-ylmethoxy)carbonyl)amino)ethyl)phenoxycarbonyl)-3-phenylsydnonimine* **393** (585 mg, 5.51 mmol, 78%) as a translucent yellow solid: R_f 0.81 (90:10, CH_2Cl_2:MeOH, UV/cerium phosphomolybdate); **m.p.** 46-49 °C; $[\alpha]_D^{20}$ +23.8, (*c* = 0.6, $CHCl_3$); ν_{max} (thin film)/cm^{-1} 2931, 2853, 1728, 1669, 1616, 1552, 1449, 1364, 1260, 1169, 1085, 1024, 799, 733, 598; **^{1}H NMR** (500 MHz; $CDCl_3$) δ 8.14 (1H, s, C*H*-30), 7.81 (2H, d, *J* 8.2 Hz, C*H*-32,36), 7.68-7.64 (3H, m, C*H*-34,44,47), 7.60 (2H, t, *J* 8.0 Hz, C*H*-33, 35), 7.47-7.44 (2H, m, C*H*-41,50), 7.32-7.21 (4H, m, C*H*-42,43,48,49), 7.18 (2H, d, *J* 8.4 Hz, C*H*-23, 27), 7.07 (2H, d, *J* 8.4 Hz, C*H*-24,26), 6.77

(1H, s, *br*, N*H*-amide), 6.12 (1H, d, *J* 7.6, N*H*-Fmoc), 4.74 (1H, dd, *J* 16.9, 8.5 Hz, C*H*-20), 4.61-4.57 (1H, m, C*H*-4), 4.37 (1H, dd, *J* 14.0, 5.7 Hz, C*H*-11), 4.28 (1H, dd, *J* 13.0, 11.0 Hz, CH_AH_B-38), 4.15-4.09 (2H, m, CH_AH_B-38, C*H*-39), 3.39-3.12 (1H, m, CH_AH_B-18), 3.03-2.94 (3H, m, CH_AH_B-18, CH_2-21), 2.41-2.28 (2H, m, CH_2-6), 2.15-2.06 (1H, m, CH_AH_B-5), 1.90-1.80 (1H, m, CH_AH_B-5) and 1.59-1.05 (33H, m, 3 × CH_3-1, 3 × CH_3-9, 3 × CH_3-14, CH_2-15, CH_2-16, CH_2-17); **^{13}C NMR** (125 MHz; $CDCl_3$) δ 175.5 (quat., C-29), 174.5, 173.6, 172.5, 172.4 (quat. × 4, C-3,7,12,19), 160.1 (quat., C-28), 157.7 (quat., C-10), 157.4 (quat., C-37), 150.9 (quat., C-25), 144.0, 143.6 (quat. × 2, C-40,51), 141.1 (quat. × 2, C-45,46), 133.8 (quat., C-22), 133.7 (quat., C-31), 133.2 (*C*H, C-34), 130.6 (*C*H × 2, C-33,35), 130.1 (*C*H × 2, C-23,27), 127.7, 127.6 (*C*H × 2, C-43,48), 127.4, 127.3 (*C*H × 2, C-42,49), 125.2, 125.3 (*C*H × 2, C-41,50), 122.0 (*C*H × 2, C-24,26), 121.8 (*C*H × 2, C-32,36), 119.8 (*C*H × 2, C-44,47), 103.3 (*C*H, C-30), 82.3, 80.9, 80.3 (quat. × 3, C-2,8,13), 67.7 (*C*H_2, C-38), 56.7 (*C*H, C-20), 53.0 (*C*H, C-11), 52.4 (*C*H, C-4), 46.7 (*C*H, C-39), 39.9 (*C*H_2, C-18), 38.5 (*C*H_2, C-21), 32.7 (*C*H_2, C-15), 31.6 (*C*H_2, C-6), 29.8 (*C*H_2, C-5), 29.3 (*C*H_2, C-16), 28.0 (3 × CH_3 × 3, C-1, C-9, C-14), 22.8 (*C*H_2, C-8); ***m/z*** (ES$^+$) 1060 ([M+H]$^+$, 100%); **HRMS** *m/z* (ES$^+$) calcd. for $C_{57}H_{70}N_7O_{13}S$ [M+H]$^+$ requires 1060.5010, found 1060.5026.

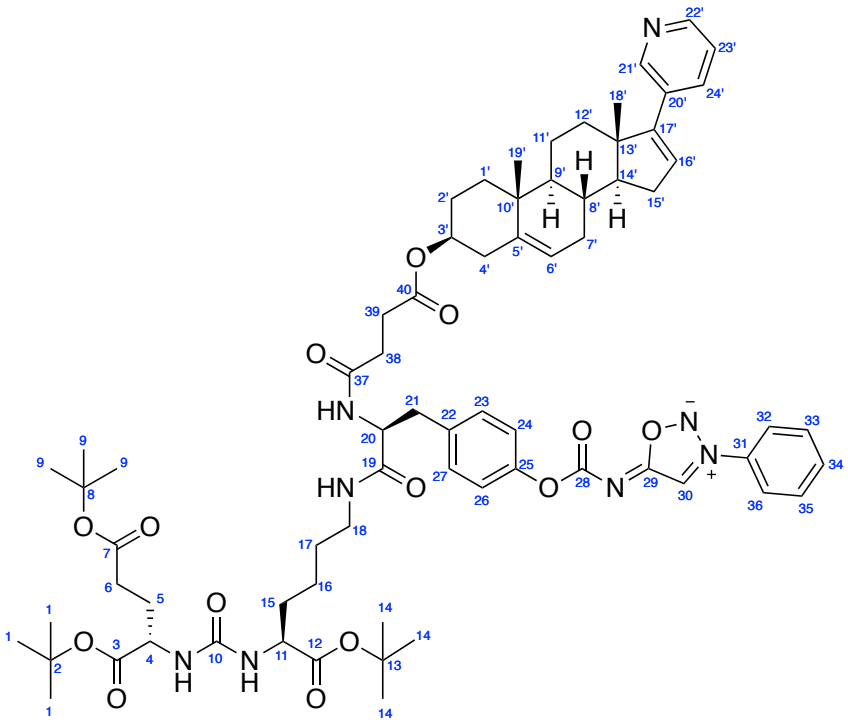
N-((4-(((2*S*)-2-Amino-2-((((5*S*)-5-((((2*S*)-1,5-*bis*(*tert*-butoxy)-1,5-dioxopentan-2-yl)carbamoyl)amino)-6-(*tert*-butoxy)-6-oxohexyl]carbamoyl)ethyl)-phenoxycarbonyl)-3-phenylsydnonimine, 394



Piperidine (100 µL, 1.01 mmol) was added to a solution of Fmoc amine **393** (122 mg, 0.12 mmol) in dry DMF (5 mL) cooled in an ice-bath. After addition, the ice-bath was removed and the solution stirred for 15 min. The solvent was evaporated under reduced pressure to yield a yellow residue. The residue suspended in dichloromethane and adsorbed onto silica gel. Purification by silica gel chromatography eluting with dichloromethane and methanol (100:0 to 90:0) provided amine **394** (87 mg, 0.10 mmol, 90%) as a pale yellow solid: R_f 0.46 (90:10, CH_2Cl_2:MeOH, UV/cerium phosphomolybdate); **m.p.** 74-76 °C; $[\alpha]_D^{20}$ -26.1, (*c* = 0.3, $CHCl_3$); ν_{max} (thin film)/cm^{-1} 2976, 2935, 1728, 1652, 1585, 1508, 1455, 1367, 1281,, 1257, 1211, 1191,

1155, 974, 846; **^{1}H NMR** (500 MHz; $CDCl_3$) δ 8.53 (1H, s, C*H*-30), 7.91 (2H, d, *J* 7.7 Hz, C*H*-32,36), 7.69 (1H, tt, *J* 7.6, 1.4 Hz, C*H*-34), 7.63 (2H, t, *J* 8.0, C*H*-33,35), 7.36 (1H, s, *br*, N*H*-amide), 7.20 (2H, d, *J* 8.7 Hz, C*H*-23,27), 7.11 (2H, d, *J* 8.7 Hz, C*H*-24,26), 5.76 (1H, d, *J* 8.9, N*H*-urea-Lys), 5.35 (1H, d, *J* 7.0, N*H*-urea-Glu), 4.28-4.20 (2H, m, C*H*-4, 11), 3.65 (1H, t, *J* 5.6 Hz, C*H*-20), 3.51 (1H, dd, *J* 13.5, 6.0 Hz, CH_AH_B-21), 3.19 (1H, dd, *J* 13.7, 6.2 Hz, CH_AH_B-18), 2.98 (1H, dd, *J* 13.0, 4.9 Hz, CH_AH_B-21), 2.93 (1H, dd, *J* 13.6, 5.2 Hz, CH_AH_B-18), 2.25-2.16 (2H, m, CH_2-17), 2.01-1.94 (1H, m, CH_AH_B-5), 1.81-1.67 (2H, m, CH_AH_B-5, CH_AH_B-6), 1.53-1.20 (32H, m, 3 × CH_3-1, 3 × CH_3-9, 3 × CH_3-14, CH_AH_B-6, CH_2-15, CH_2-16); **^{13}C NMR** (125 MHz; $CDCl_3$) δ 175.9 (quat., C-29), 173.9, 173.1, 172.2 (quat., × 4, C-3,7,12,19), 160.9 (quat., C-28), 157.5 (quat., C-10), 150.8 (quat., C-25), 133.9 (quat., C-22), 133.8 (quat., C-31), 133.1 (*C*H, C-4)**,** 130.8 (*C*H × 2, C-33,35), 130.5 (*C*H × 2, C-23, 27), 121.9 (*C*H × 2, C-24,26), 121.8 (*C*H × 2, C-32,36), 104.1 (*C*H, C-30), 81.6, 81.2, 80.5 (quat., × 3, C-2,8,13)**,** 55.7 (*C*H, C-20), 52.8 (*C*H, C-4), 52.8 (*C*H, C-11), 39.7 (CH_2, C-18), 38.6 (CH_2, C-21), 32.1 (CH_2, C-6), 31.6 (CH_2, C-17), 28.5 (CH_2, C-5), 28.4 (CH_2, C-15), 28.0 (3 × CH3 × 3, C-1,9,14), 22.5 (CH_2, C-16); ***m/z*** (ES^+) 838 ($[M+H]^+$, 100%); **HRMS** *m/z* (ES^+) calcd. for $C_{42}H_{60}N_7O_{11}$ $[M+H]^+$ requires 838.4345, found 838.4346.

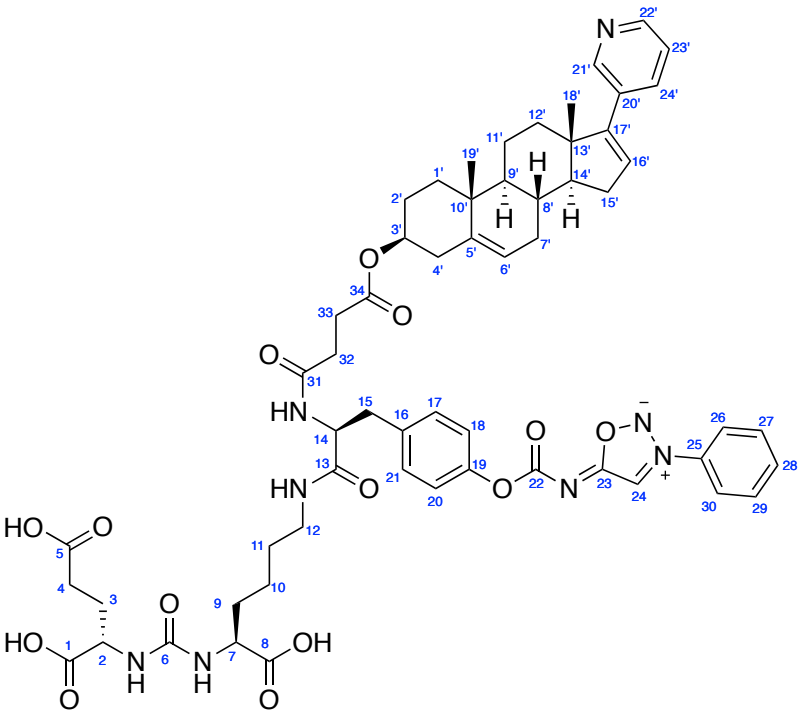
(OtBu)$_3$-PSMA-abiraterone conjugate, 395



Following general procedure C with HATU (459 mg, 1.21 mmol), abiraterone hemisuccinate **352** (329 mg, 0.60 mmol), amine **394** (334 mg, 0.40 mmol) and diisopropylethylamine (distilled, 208 mg, 275 µL, 1.61 mmol). Purification by silica gel chromatography, eluting with

dichloromethane and methanol (98:2) provided *(OtBu)$_3$-PSMA-abiraterone* conjugate **395** (464 mg, 0.37 mmol, 91%) as a translucent yellow solid: $\boldsymbol{R_f}$ 0.76 (90:10, CH_2Cl_2:MeOH, UV/cerium phosphomolybdate); **m.p.** 109-111 °C; $[\boldsymbol{\alpha}]_{\mathbf{D}}^{\mathbf{20}}$+7.7, (*c* = 0.7, $CHCl_3$); $\boldsymbol{\nu_{max}}$ (thin film)/cm^{-1} 3377, 3054, 2984, 2305, 1726, 1641, 1589, 1554, 1509, 1422, 1368, 1265, 1217, 1192, 1155, 972, 996, 846, 739; **^{1}H NMR** (500 MHz; $CDCl_3$) δ 8.61 (1H, s, C*H*-21′), 8.46 (1H, s, C*H*-24′), 8.25 (1H, s, C*H*-30), 7.87 (2H, d, *J* 7.8 Hz, C*H*-33,36), 7.70-7.62 (4H, m, C*H*-33,34,35,22′), 7.25 (1H, s, *br*, C*H*-23′), 7.19-7.09 (4H, m, C*H*-23,24, 26,27), 6.04 (1H, d, *J* 5.8, N*H*-urea-Lys), 6.00-6.98 (1H, m, C*H*-16′), 5.23 (1H, d, *J* 3.7 Hz, C*H*-6′), 5.11-4.98 (1H, m, C*H*-20), 4.64-4.48 (2H, m, C*H*-11,3′), 4.39 (1H, m, C*H*-4), 3.46-3.99 (1H, m, CH_AH_B-18), 3.07-2.88 (2H, m, CH_AH_B-18, CH_2-21), 2.47-2.20 (9H, m, CH_2-6, CH_2-38, CH_2-29, CH_2-1′, CH_AH_B-12′), 2.17-2.09 (1H, m, CH_AH_B-5), 2.07-1.95 (2H, m, CH_AH_B-7′, CH_AH_B-12′), 1.91-1.14 (43H, m, 3 × CH_3-1, CH_AH_B-5, 3 × CH_3-9, 3 × CH_3-14, CH_2-15,16, CH_AH_B-17, CH_2-2′, CH_AH_B-4′, CH_AH_B-7′, CH_2-9′,11′,15′) and 1.10-1.97 (9H, m, CH_AH_B-17, CH_AH_B-4′, C*H*-14, CH_3-18′,19′); **^{13}C NMR** (125 MHz; $CDCl_3$) δ 175.7 (quat., C-29), 174.7, 174.7, 172.8, 172.6, 172.3, 171,7 (quat., × 6, C-3,7,12,19,37,40), 160.2 (quat., C-28), 157.7 (quat., C-10), 151.4 (quat., C-17′), 150.9 (quat., C-25), 147.1 (*C*H, C-24′), 147.1 (*C*H, C-21′), 140.0 (quat., C-5′), 134.3 (*C*H, C-22′), 133.9 (quat., C-22), 133.7 (quat., C-31), 133.3 (quat., C-20′), 133.1 (*C*H, C-34), 130.6 (*C*H × 2, C-23,35), 130.1 (*C*H × 2, C-23,27), 129.8 (*C*H, C-16′), 123.4 (*C*H, C-23′), 122.1 (*C*H, C-6′), 122.0 (*C*H × 2, C-24,26), 121.8 (*C*H × 2, C-32,36), 103.6 (*C*H, C-30), 82.5, 80.8, 80.4 (quat., × 6, C-2,8,13), 73.9 (*C*H, C-3′), 57.4 (*C*H, C-9′), 54.8 (*C*H, C-20), 53.0 (*C*H, C-11), 52.3 (*C*H, C-4), 50.1 (*C*H, C-14′), 47.3 (quat., C-13′), 39.8 (*C*H$_2$, C-18), 38.3 (*C*H$_2$, C-21), 38.1 (*C*H$_2$, C-1′), 36.8 (*C*H$_2$, C-4′), 36.7 (quat., C-10′), 35.2 (*C*H$_2$, C-7′), 32.6 (*C*H$_2$, C-15), 31.5 (*C*H$_2$, C-15′), 31.8 (*C*H$_2$, C-12′), 31.7 (*C*H$_2$, C-6), 30.3 (*C*H$_2$, C-8′), 30.0 (*C*H$_2$, C-16), 29.7 (*C*H$_2$ × 2, C-38,39), 29.0 (*C*H$_2$, C-5), 28.1, 28.1, 28.0 (3 × CH3 × 3, C-1,9,14), 27.7 (*C*H$_2$, C-2′), 22.4 (*C*H$_2$, C-17), 20.7 (*C*H$_2$, C-11′), 19.2 (*C*H$_3$, C-19′), 16.6 (*C*H$_3$, C-18′); ***m/z*** (ES$^+$) 1291 ([M+Na]$^+$, 15%), 1269 ([M+H]$^+$, 100%); **HRMS** *m/z* (ES$^+$) calcd. for $C_{70}H_{93}N_8O_{14}$ [M+H]$^+$ requires 1269.6806, found 1269.6777.

PSMA-abiraterone conjugate, 396



Trifluoroacetic acid (1 mL, 13.1 mmol) was added to a solution of amide **395** (100 mg, 0.08 mmol) in dichloromethane (4 mL) cooled in an ice-bath. The solution was stirred for 18 h. The solvent was removed under reduced pressure and the residue evaporated from toluene, methanol, and dichloromethane. The residue was triturated with cold acetonitrile to furnish *PSMA-abiraterone* conjugate **396** (76 mg, 0.07, 88%) as a white solid: $\boldsymbol{R_f}$ 0.00 (90:10, CH_2Cl_2:MeOH, UV/cerium phosphomolybdate); **m.p.** 95 °C (decomp.); $\boldsymbol{\nu_{max}}$ (thin film)/cm^{-1} 2923, 2852, 1716, 1674, 1456, 1377, 1157, 895′ **^{1}H NMR** (500 MHz, d_6-DMSO) δ 8.75 (1H, s, *br*, C*H*-21′), 8.64-8.61 (2H, m, C*H*-24,24′), 8.23 (1H, d, *J* 7.5 Hz, C*H*-22′), 8.16 (1H, d, *J* 8.4 Hz, N*H*-succinamide), 8.05 (2H, d, *J* 7.6 Hz, C*H*-26, 30), 7.93 (1H, t, *J* 7.1 Hz, N*H*-amide), 7.78-7.69 (4H, m, C*H*-27, 28, 29, 23′), 7.21 (2H, d, *J* 8.3 Hz, C*H*-18,20), 7.01 (2H, d, *J* 8.3 Hz, C*H*-17, 20), 6.34-6.26 (3H, m, 2 × N*H*-urea, C*H*-16′), 5.36 (1H, d, *J* 3.9, C*H*-6′), 4.45-4.37 (2H, m, C*H*-2, 7), 3.06-2.92 (3H, m, CH_2-12, CH_AH$_B$-15), 2.74 (1H, dd, *J* 13.1, 9.6 Hz, CH$_A$*H*$_B$-15), 2.44-2.15 (9H, m, CH_2-4,32,33, CH_AH$_B$-12), 2.12-1.44 (16H, m, CH_2-3, CH_2-9, CH_2-2′, CH_AH$_B$-4′, CH_AH$_B$-7′, C*H*-8′, C*H*-9′, CH_2-11′, CH$_A$*H*$_B$-12′, CH_2-15′), 1.41-1.31 (3H, m, CH_2-11, CH$_A$*H*$_B$-7′), 1.25 (2H, dt, *J* 7.5, 6.9, CH_2-10), 1.07-0.99 (8H, m, CH$_A$*H*$_B$-4′, C*H*-14′, CH_3-18′,19′); **^{13}C NMR** (125 MHz, d_6-DMSO) δ 175.3 (quat., C-23), 175.2, 174.8, 174.3, 172.4, 171.4, 171.3 (quat. × 6, C-1,5,7,13,31,34), 159.3 (quat., C-22), 157.9 (quat., C-6), 151.0 (quat., C-19), 149.7 (quat., C-17′), 143.7 (*C*H, C-24′), 142.8 (*C*H, C-21′), 140.4 (quat., C-5′), 139.3 (*C*H, C-22′), 134.9 (quat., C-16), 134.6 (quat., C-20′), 134.1 (quat., C-25), 133.6 (*C*H, C-28), 132.9 (*C*H, C-16′), 130.9 (*C*H × 2, C-27,29), 130.3 (*C*H × 2, C-18,20), 126.2 (*C*H, C-23′), 122.9

(*C*H × 2, C-26,30), 122.4 (*C*H, C-6′), 121.9 (*C*H × 2, C-17,21), 105.3 (*C*H, C-24), 73.8 (*C*H, C-3′), 57.9 (*C*H, C-9′), 54.7 (*C*H, C-14), 52.8, 52.2 (*C*H × 2, C-2,7), 50.1 (*C*H, C-14′), 47.2 (quat., C-13′), 39.0 (CH$_2$, C-12), 38.2 (CH$_2$, C-1′), 37.8 (CH$_2$, C-15), 36.9 (quat., C-10′), 36.8 (CH$_2$, C-4′), 34.7 (CH$_2$, C-7′), 32.3 (CH$_2$, C-9), 32.0 (CH$_2$, C-12′), 31.4 (CH$_2$, C-4), 30.4 (CH$_2$, C-15′), 29.9 (*C*H, C-8′), 29.2 (CH$_2$, C-11), 28.2 (*C*H2 × 2, C-32,33), 28.1 (CH$_2$, C-3), 27.8 (CH$_2$, C-2′), 23.2 (CH$_2$, C-10), 21.0 (CH$_2$, C-11′), 19.5 (CH$_3$, C-19′) and 16.6 (CH$_3$, C-18′); ***m/z*** (ES^+) 1101 ($[M+H]^+$, 100%); **HRMS** *m/z* (ES^+) calcd. for $C_{58}H_{69}N_8O_{14}$ $[M+H]^+$ requires 1101.4933, found 1101.4945.

6.7 MATERIALS AND METHODS

Procedure adapted from that detailed in nitrite/nitrate determination kit purchased from Sigma Aldrich. All solutions used were from this kit. Buffer solution (pH 7.6, containing 6 mmol GST, 4557 U mL−1, superoxide dismutase *or* pH 7.6 buffer) was added to the substrate (1 mmol). The mixture was incubated at room temperature for 1 h. Griess reagent A (1% sulfanilamide, 5% H_3PO_4 in distilled H_2O) was added and incubated for 5 min. Griess reagent B (*N*-1-naphthyl)ethylenediamine dihydrochloride in distilled H_2O) was added and the mixture incubated for 10 min. The final concentration of substrate was 400 μM. UV absorbance at 568 nm was measured using a Multiskan FC Microplate Photometer and calibrated using a standard curve prepared from a standard solution of $NaNO_2$ to give nitrite concentration. Measurements were made in triplicate.

REFERENCES

(1) Altshuller, A. P.; Bufalini, J. J. *Environ. Sci. Technol.* **1971**, *5*, 39–64.

(2) Barton, C. H.; Ni, Z.; Vaziri, N. C. *Am. J. Hypertens.* **2003**, *16*, 1043–1048.

(3) Dawson, T. M.; Dawson, V. L. *Neuroscientist* **1995**, *1*, 7–18.

(4) Ignarro, L. J. *Nitric Oxide, Biology and Pathobiology*; Academic Press: San Diego, 1000.

(5) Kröncke, K.-D.; Fehsel, K.; Kolb-Bachofen, V. *Nitric Oxide* **1997**, *1*, 107–112.

(6) Koshland, D. E. *Science* **1992**, *258*, 1861.

(7) Recipients:; Furchgott, R. F.; Ignarro, L. J.; Murad, F. 1998.

(8) Zacharia, I. G.; Deen, W. M. *Ann. Biomed. Eng.* **2005**, *33*, 214–222.

(9) Andrew, P. J.; Mayer, B. *Cardiovasc. Res.* **1999**, *43*, 521–531.

(10) Gray, H. *Anatomy of the human body*; Warren, H. L., Ed.; 20th ed.; Bartleby: Philadelphia, 2000.

(11) Ignarro, L. J.; Bush, P. A.; Buga, G. M.; Wood, K. S.; Fukuto, J. M.; Rajfer, J. *Biochem. Biophys. Res. Commun.* **1990**, *170*, 843–850.

(12) Furchgott, R. F.; Zawadzki, J. V. *Nature* **1980**, *288*, 373–376.

(13) Parker, J. O.; Vankoughnett, K. A.; Farrell, B. *Am. J. Cardiol.* **1986**, *57*, 1–5.

(14) Yeh, B. K.; Gosselin, A. J.; Swaye, P. S.; Larsen, P. B.; Gentsch, T. O.; Traad, E. A.; Faraldo, A. R. *Am. Heart. J.* **1977**, *93*, 610–616.

(15) Ignarro, L. J.; Buga, G. M.; Wood, K. S.; Byrns, R. E.; Chaudhuri, G. *Proc. Natl. Acad. Sci. U.S.A.* **1987**, *84*, 9265–9269.

(16) Palmer, R. M. J.; Ferrige, A. G.; Moncada, S. *Nature* **1987**, *327*, 524–526.

(17) Rall, T. W.; Sutherland, E. W. *J. Biol. Chem.* **1958**, *232*, 1056–1076.

(18) Arnold, W. P.; Mittal, C. K.; Katsuki, S.; Murad, F. *Proc. Natl. Acad. Sci. U.S.A.* **1977**, *74*, 3203–3207.

(19) Schultz, K.-D.; Schultz, K.; Schultz, G. *Nature* **1977**, *265*, 750–751.

(20) Martin, E.; Berka, V.; Tsai, A. L.; Murad, F. *Methods Enzymol.* **2005**, *396*, 478–492.

(21) Zabel, U.; Weeger, M.; La, M.; Schmidt, H. H. *Biochem. J.* **1998**, *335*, 51–57.

(22) Allerston, C. K.; Von Delft, F.; Gileadi, O. *PLoS ONE* **2013**, *8*, e57644.

(23) Stone, J. R.; Marletta, M. A. *Biochemistry* **1996**, *35*, 1093–1099.

(24) Perutz, M. F.; Rossmann,, M. G.; Cullis, A. N. N. F.; Muirhead, H.; Will, G.; North, A. C. T. *Nature* **1960**, *185*, 416–422.

(25) Atkins, P.; Overton, T.; Rourke, J.; Weller, M.; Armstrong, F. *Shriver and Atkins' Inorganic Chemistry*; Oxford University Press, 2010; p. Chapter 21.

(26) Hayton, T. W.; Legzdins, P.; Sharp, W. B. *Chem. Rev.* **2002**, *102*, 935–992.

(27) Dierks, E. A.; Hu, S.; Vogel, K. M.; Yu, A. E.; Spiro, T. G.; Burstyn, J. N. *J. Am. Chem. Soc.* **1997**, *119*, 7316–7323.

(28) Ignarro, L. J.; Ballot, B.; Wood, K. S. *J. Biol. Chem.* **1984**, *259*, 6201–6207.

(29) Vanhoutte, P. M. *Hypertension* **1989**, *13* , 658–667.

(30) Stasch, J.-P.; Pacher, P.; Evgenov, O. V *Circulation* **2011**, *123* , 2263–2273.

(31) Langford, E. J.; Wainwright, R. J.; Martin, J. F. *Arterioscler. Thromb. Vasc. Biol.* **1996**, *16*, 51–55.

(32) Hensley, L. E.; Geisbert, T. W. *Thromb. Haemostasis* **2005**, *94*, 254–261.

(33) Lee, J.-W.; Bae, S.-H.; Jeong, J.-W.; Kim, S.-H.; Kim, K.-W. *Exp. Mol. Med.* **2004**, *36*, 1–12.

(34) Vaupel, P.; Thews, O.; Hoeckel, M. *Med. Oncol.* **2001**, *18*, 243–259.

(35) Brown, J. M.; Giaccia, A. J. *Cancer Res.* **1998**, *58*, 1408–1416.

(36) Dewhirst, M. W.; Kimura, H.; Rehmus, S. W. E.; Braun, R. D.; Papahadjopoulos, D.; Hong, K.; Secomb, T. W. *Br. J. Cancer* **1996**, *74 Suppl.*, S247–S251.

(37) Vaupel, P.; Kelleher, D. K.; Hockel, M. *Semin. Oncol.* **2001**, *28*, 29–35.

(38) Overgaard, J. *J. Clin. Oncol.* **2007**, *25*, 4066–4074.

(39) Bruick, R. K. *Genes. Dev.* **2003**, *17*, 2614–2623.

(40) Ke, Q.; Costa, M. *Mol. Pharmacol.* **2006**, *70*, 1469–1480.

(41) Wang, G. L.; Jiang, B. H.; Rue, E. A.; Semenza, G. L. *Proc. Natl. Acad. Sci. U.S.A.* **1995**, *92*, 5510–5514.

(42) Carroll, V. A.; M., A. *Exp. Rev. Mol. Med.* **2005**, *7*, 1.

(43) Huang, L. E.; Gu, J.; Schau, M.; Bunn, H. F. *Proc. Natl. Acad. Sci. U.S.A.* **1998**, *95*, 7987–7992.

(44) Ivan, M.; Kondo, K.; Yang, H.; Kim, W.; Valiando, J.; Ohh, M.; Salic, A.; Asara, J. M.; Lane, W. S.; Kaelin Jr., W. G. *Science* **2001**, *292* , 464–468.

(45) Jaakkola, P.; Mole, D. R.; Tian, Y.-M.; Wilson, M. I.; Gielbert, J.; Gaskell, S. J.; Kriegsheim, A. von; Hebestreit, H. F.; Mukherji, M.; Schofield, C. J.; Maxwell, P. H.; Pugh, C. W.; Ratcliffe, P. J. *Science* **2001**, *292*, 468–472.

(46) Ebert, B. L.; Bunn, H. F. *Blood* **1999**, *94* , 1864–1877.

(47) Carmeliet, P.; Dor, Y.; Herbert, J.-M.; Fukumura, D.; Brusselmans, K.; Dewerchin, M.; Neeman, M.; Bono, F.; Abramovitch, R.; Maxwell, P.; Koch, C. J.; Ratcliffe, P.; Moons, L.; Jain, R. K.; Collen, D.; Keshet, E. *Nature* **1998**, *394*, 485–490.

(48) Sowter, H. M.; Ratcliffe, P. J.; Watson, P.; Greenberg, A. H.; Harris, A. L. *Cancer Res.* **2001**, *61*, 6669–6673.

(49) Mateo, J.; García-Lecea, M.; Cadenas, S.; Hernández, C.; Moncada, S. *Biochem. J.* **2003**, *376*, 537–544.

(50) Chandel, N. S.; Maltepe, E.; Goldwasser, E.; Mathieu, C. E.; Simon, M. C.; Schumacker, P. T. *Proc. Natl. Acad. Sci. U.S.A.* **1998**, *95*, 11715–11720.

(51) Schroedl, C.; McClintock, D. S.; Budinger, G. R. S.; Chandel, N. S. *Am. J. Physiol-Lung C* **2002**, *283*, L922–L931.

(52) Hagen, T.; Taylor, C. T.; Lam, F.; Moncada, S. *Science* **2003**, *302* , 1975–1978.

(53) Pepke-Zaba, J.; Higenbottam, T. W.; Dinh-Xuan, A. T.; Stone, D.; Wallwork, J. *Lancet* **1991**, *338*, 1173–1174.

(54) Smyth, R. L. *Thorax* **2000**, *55 Suppl.*, S51–S55.

(55) Skimming, J. W.; Bender, K. A.; Hutchison, A. A.; Drummond, W. H. *J. Pediatr.* **1997**, *130*, 225–230.

(56) Fukumura, D.; Kashiwagi, S.; Jain, R. K. *Nat. Rev. Cancer* **2006**, *6*, 521–534.

(57) Coulter, J. A.; Page, N. L.; Worthington, J.; Robson, T.; Hirst, D. G.; McCarthy, H. O. *J. Gene. Med.* **2010**, *12*, 755–765.

(58) Wink, D. A.; Cook, J. A.; Krishna, M. C.; Hanbauer, I.; Degraff, W.; Gamson, J.; Mitchell, J. B. *Arch. Biochem. Biophys.* **1995**, *319*, 402–407.

(59) Wink, D. A.; Hanbauer, I.; Krishna, M. C.; DeGraff, W.; Gamson, J.; Mitchell, J. B. *Proc. Natl. Acad. Sci. U.S.A.* **1993**, *90*, 9813–9817.

(60) Struck, A. T.; Hogg, N.; Thomas, J. P.; Kalyanaraman, B. *FEBS Lett.* **1995**, *361*, 291–294.

(61) Godoy, L. C.; Anderson, C. T. M.; Chowdhury, R.; Trudel, L. J.; Wogan, G. N. *Proc. Natl. Acad. Sci. U.S.A.* **2012**.

(62) Stupp, R.; Mason, W. P.; Van den Bent, M. J.; Weller, M.; Fisher, B.; Taphoorn, M. J. B.; Belanger, K.; Brandes, A. A.; Marosi, C.; Bogdahn, U.; Curschmann, J.; Janzer, R. C.; Ludwin, S. K.; Gorlia, T.; Allgeier, A.; Lacombe, D.; Cairncross, J. G.; Eisenhauer, E.; Mirimanoff, R. O. *New Eng. J. Med.* **2005**, *352*, 987–996.

(63) Eyler, C. E.; Wu, Q.; Yan, K.; MacSwords, J. M.; Chandler-Militello, D.; Misuraca, K. L.; Lathia, J. D.; Forrester, M. T.; Lee, J.; Stamler, J. S.; Goldman, S. A.; Bredel, M.; McLendon, R. E.; Sloan, A. E.; Hjelmeland, A. B.; Rich, J. N. *Cell* **2011**, *146*, 53–66.

(64) Thomsen, L. L.; Miles, D. W.; Happerfield, L.; Bobrow, L. G.; Knowles, R. G.; Moncada, S. *Br. J. Cancer* **1995**, *72*, 41–44.

(65) Vakkala, M.; Kahlos, K.; Lakari, E.; Pääkkö, P.; Kinnula, V.; Soini, Y. *Clin. Cancer Res.* **2000**, *6*, 2408–2416.

(66) Loibl, S.; Von Minckwitz, G.; Weber, S.; Sinn, H.-P.; Schini-Kerth, V. B.; Lobysheva, I.; Nepveu, F.; Wolf, G.; Strebhardt, K.; Kaufmann, M. *Cancer* **2002**, *95*, 1191–1198.

(67) Xu, W.; Liu, L. Z.; Loizidou, M.; Ahmed, M.; Charles, I. G. *Cell Res.* **2002**, *12*, 311–320.

(68) Burney, S.; Caulfield, J. L.; Niles, J. C.; Wishnok, J. S.; Tannenbaum, S. R. *Mutat. Res.-Fund. Mol. M.* **1999**, *424*, 37–49.

(69) Kelman, D. J.; Christodoulou, D.; Wink, D. A.; Keefer, L. K.; Srinivasan, A.; Dipple, A. *Carcinogenesis* **1997**, *18* , 1045–1048.

(70) Niles, J. C.; Wishnok, J. S.; Tannenbaum, S. R. *Nitric Oxide* **2006**, *14*, 109–121.

(71) Huie, R. E.; Padmaja, S. *Free Radical Res. Commun.* **1993**, *18*, 195–199.

(72) Traylor, T. G.; Sharma, V. A. *Biochemistry* **1992**, *31*, 2847–2849.

(73) McCord, J. M.; Fridovich, I. *Free Radical Biol. Med.* **1988**, *5*, 363–369.

(74) Yun, B. H.; Geacintov, N. E.; Shafirovich, V. *Chem. Res. Tox.* **2011**, *24*, 1144–1152.

(75) Pieper, A. A.; Verma, A.; Zhang, J.; Snyder, S. H. *Trends Pharmacol. Sci.* **1999**, *20*, 171–181.

(76) Langelier, M.-F.; Planck, J. L.; Roy, S.; Pascal, J. M. *J. Biol. Chem.* **2011**, *286*, 10690–10701.

(77) Yu, X.; Hathout, Y.; Fenselau, C.; Sowder 2nd, R. C.; Henderson, L. E.; Rice, W. G.; Mendeleyev, J.; E., K. *Chem. Res. Toxicol.* **1995**, *8*, 586–590.

(78) Men, L.; Roginskaya, M.; Zou, Y.; Wang, Y. *Rapid. Commun. Mass Sp.* **2007**, *21*, 2743–2749.

(79) Jones, M. K.; Tsugawa, K.; Tarnawski, A. S.; Baatar, D. *Biochem. Biophys. Res. Commun.* **2004**, *318*, 520–528.

(80) Wang, P. G.; Xian, M.; Tang, X.; Wu, X.; Wen, Z.; Cai, T.; Janczuk, A. J. *Chem. Rev.* **2002**, *102*, 1091–1134.

(81) Cachofeiro, V.; Sakakibara, T.; Nasjletti, A. *Hypertension* **1992**, *19*, 138–145.

(82) Dimmeler, S.; Fleming, I.; Fisslthaler, B.; Hermann, C.; Busse, R.; Zeiher, A. M. *Nature* **1999**, *399*, 601–605.

(83) Dhein, S.; Salameh, A. *Circulation* **1999**, *100* , 1011–1015.

(84) Boschan, R.; Merrow, R. T.; Van Dolah, R. W. *Chem. Rev.* **1955**, *55*, 485–510.

(85) Doyle, M. P.; Terpstra, J. W.; Pickering, R. A.; LePoire, D. M. *J. Org. Chem.* **1983**, *48*, 3379–3382.

(86) Lijinsky, W. *Chemistry and Biology of N-Nitroso Compounds*; Cambridge University Press: New York, 1992.

(87) Alston, T. A.; Porter, D. J.; Bright, H. J. *J. Biol. Chem.* **1985**, *260*, 4069–4074.

(88) Challis, B. C.; Challis, J. A. In *Amino, Nitroso and Nitro Compounds and Their Derivatives (1982)*; John Wiley & Sons, Ltd., 2010; pp. 1151–1223.

(89) Al-Sa'Doni, H.; Ferro, A. *Clin. Sci.* **2000**, *98*, 507–520.

(90) Chakrapani, H.; Bartberger, M. D.; Toone, E. J. *J. Org. Chem.* **2009**, *74*, 1450–1453.

(91) Gooden, D. M.; Chakrapani, H.; Toone, E. J. *Curr. Top. Med. Chem.* **2005**, *7*, 687–705.

(92) Gasco, A.; Fruttero, R.; Sorba, G.; Di Stilo, A.; Calvino, R. *Pure Appl. Chem.* **2004**, *76*, 973–981.

(93) Kankaanranta, H.; Rydell, E.; Petersson, A.-S.; Holm, P.; Moilanen, E.; Corell, T.; Karup, G.; Vuorinen, P.; Pedersen, S. B.; Wennmalm, Å. R.; Metsä-Ketelä, T. *Br. J. Pharmacol.* **1996**, *117*, 401–406.

(94) Beal, E. N.; Turnbull, K. *Synth. Commun.* **1992**, *22*, 673–676.

(95) Kato, M.; Nishino, S.; Ohno, M.; Fukuyama, S.; Kita, Y.; Hirasawa, Y.; Nakanishi, I.; Takasugi, H.; Sakane, K. *Bioorg. Med. Chem. Lett.* **1996**, *6*, 33–38.

(96) DeMaster, E. G.; Raij, L.; Stephen, L. A.; Weir, E. K. *Biochem. Biophys. Res. Commun.* **1989**, *163*, 527–533.

(97) Zhang, Q.; Kulczynska, A.; Webb, D. J.; Megson, I. L.; Botting, N. P. *Chem. Commun.* **2013**, *49*, 1389–1391.

(98) King, S. B. *Free Radical Biol. Med.* **2004**, *37*, 737–744.

(99) Samuni, Y.; Samuni, U.; Goldstein, S. *Biochem. Biophys. Acta* **2012**, *1820*, 1560–1566.

(100) Lange, K.; Koenig, A.; Roegler, C.; Seeling, A.; Lehmann, J. *Bioorg. Med. Chem. Lett.* **2009**, *19*, 3141–3144.

(101) Bolla, M.; Almirante, N.; Benedini, F. *Curr. Top. Med. Chem.* **2005**, *5*, 707–720.

(102) Philips, H. C. Preparation of Nitrocellulose **1950**, US 2510834.

(103) Wheeler, W. H.; Whittaker, H.; Pike, H. H. M. *J. Inst. Fuel* **1947**, *20*, 137–156/159.

(104) Diplock, B. R.; Lofts, D. L.; Grimston, R. A. *Aeronaut. Soc.* **1953**, *57*, 19–28.

(105) Grewer, T.; Rogers, R. L. *Thermochim. Acta* **1993**, *225*, 289–301.

(106) Serkov, I. V; Bezuglov, V. V *Chem. Nat. Comp.* **2008**, *44*, 67–68.

(107) Oldham, J. W. H.; Rutherford, J. K. *J. Am. Chem. Soc.* **1932**, *54*, 366–378.

(108) Ferris, A. F.; McLean, K. W.; Marks, I. G.; Emmons, W. D. *J. Am. Chem. Soc.* **1953**, *75*, 4078.

(109) Fieser, L. F.; Doering, W. von E. *J. Am. Chem. Soc.* **1946**, *68*, 2252–2253.

(110) Baker, J. W.; Easty, D. M. *Nature* **1950**, *166*, 156.

(111) Cainelli, G.; Manescalchi, F.; Martelli, G.; Panunzio, M.; Plessi, L. *Tetrahedron Lett.* **1985**, *26*, 3369–3372.

(112) Welsch, T.; Tran, H.-A.; Witulski, B. *Org. Lett.* **2010**, *12*, 5644–5647.

(113) Hwu, J. R.; Vyas, K. A.; Patel, H. V; Lin, C.-H.; Yang, J.-C. *Synthesis* **1994**, 471–473.

(114) Lucas, G. R.; Hammett, L. P. *J. Am. Chem. Soc.* **1942**, *64*, 1928–1937.

(115) Baker, J. W. *J. Chem. Soc.* **1934**, 987–992.

(116) Capellos, C.; Fisco, W. J.; Ribaudo, C.; Hogan, V. D.; Campisi, J.; Murphy, F. X.; Castorina, T. C.; Rosenblatt, D. H. *Int. J. Chem. Kinet.* **1984**, *16*, 1027–1051.

(117) Ignarro, L. J.; Lippton, H.; Edwards, J. C.; Baricos, W. H.; Hyman, A. L.; Kadowitz, P. J.; Gruetter, C. A. *J. Pharmacol. Exp. Ther.* **1981**, *218*, 739–749.

(118) Tsou, P.-S.; Page, N.; Lee, S.; Fung, S.; Keung, W.; Fung, H.-L. *AAPS J.* **2011**, *13*, 548–555.

(119) Doel, J. J.; Godber, B. L. J.; Goult, T. A.; Eisenthal, R.; Harrison, R. *Biochem. Biophys. Res. Commun.* **2000**, *270*, 880–885.

(120) Keen, J. H.; Habig, W. H.; Jakoby, W. B. *J. Biol. Chem.* **1976**, *251*, 6183–6188.

(121) Zheng, H.; Wisedchaisri, G.; Gonen, T. *Nature* **2013**, *497*, 647–651.

(122) Yeates, R. A. *Arzneim.-Forsch/Drug Res.* **1992**, 1314–1317.

(123) Artz, J. D.; Toader, V.; Zavorin, S. I.; Bennett, B. M.; Thatcher, G. R. J. *Biochemistry* **2001**, *40*, 9256–9264.

(124) Bertinaria, M.; Rolando, B.; Giorgis, M.; Montanaro, G.; Marini, E.; Collino, M.; Benetti, E.; Daniele, P. G.; Fruttero, R.; Gasco, A. *Eur. J. Med. Chem.* **2012**, *54*, 103–112.

(125) Qin, Z.; Luo, J.; VandeVrede, L.; Tavassoli, E.; Fa', M.; Teich, A. F.; Arancio, O.; Thatcher, G. R. J. *J. Med. Chem.* **2012**, *55*, 6784–6801.

(126) Caravaggi, A. M.; Sardi, A.; Balodi, E.; Di Francesco, G. F.; C., L. T. *Arch. Int. Pharmocodyn. Ther.* **1977**, *226*, 139–148.

(127) Kawashima, Y.; Ikemoto, T.; Horiguchi, A.; Hayashi, M.; Matsumoto, K.; Kawarasaki, K.; Yamazaki, R.; Okuyama, S.; Hatayama, K. *J. Med. Chem.* **1993**, *36*, 815–819.

(128) Gasco, A.; Boulton, A. J. *Adv. Heterocyl. Chem.* **1981**, *29*, 251–340.

(129) Wieland, H.; Semper, L. *Leibigs. Ann. Chem.* **1908**, *358*, 36–70.

(130) Godovikova, T. I.; Golova, S. P.; Streienko, Y. A.; Antipin, M. Y.; Struchkov, Y. T.; Khmeĺnitskii, L. I. *Mendeleev Commun.* **1994**, *4*, 7–9.

(131) Thakore, A. N.; Buchshriber, J.; Oehlschlager, A. C. *Can. J. Chem.* **1973**, *51*, 2406–2414.

(132) Emmons, W. D.; Freeman, J. P. *J. Org. Chem.* **1957**, *22*, 456–457.

(133) Armani, V.; Dell'Erba, C.; Marino, N.; Petrillo, G.; Tavani, C. *Tetrahedron* **1997**, *53*, 1751–1758.

(134) Boulton, A. J.; Hadjimihalakis, P.; Katritzky, A. R.; Hamid, A. M. *J. Chem. Soc. C.* **1969**, 1901–1903.

(135) Grundman, G.; Grünanget, P. *The Nitrile Oxides*; Springer-Verlag: Berlin and New York, 1971.

(136) Mukaiyama, T.; Nambu, H.; Okamoto, M. *J. Org. Chem.* **1962**, *27*, 3651–3654.

(137) Whitney, R. A.; Nicholas, E. S. *Tetrahedron Lett.* **1981**, *22*, 3371–3374.

(138) Fruttero, R.; Sorba, G.; Ermondi, G.; Lolli, M.; Gasco, A. *Il Farmaco* **1997**, *52*, 405–410.

(139) Feelisch, M.; Schönafingeri, K.; Noack, H. *Bio. Pharmacol.* **1992**, *44*, 1149–1157.

(140) Feelisch, M.; Ostrowski, J.; Noack, E. *J. Cardiovascl. Pharmacol.* **1989**, *14*, S13–22.

(141) Fruttero, R.; Boschi, D.; Di Stilo, A.; Gasco, A. *J. Med. Chem.* **1995**, 4944–4949.

(142) Boschi, D.; Di Stilo, A.; Cena, C.; Lolli, M.; Fruttero, R.; Gasco, A. *Pharm. Res.* **1997**, 1750–1758.

(143) Cena, C.; Visentin, S.; Di Stilo, A.; Boschi, D.; Fruttero, R.; Gasco, A. *Pharm. Res.* **2001**, 157–165.

(144) Mu, L.; Feng, S. S.; Go, M. L. *Chem. Pharm. Bull.* **2000**, *48*, 808–816.

(145) Turnbull, C. M.; Cena, C.; Fruttero, R.; Gasco, A.; Rossi, A. G.; Megson, I. L. *Br. J. Pharmacol.* **2006**, *148*, 517–526.

(146) Lolli, M. L.; Cena, C.; Medana, C.; Lazzarato, L.; Morini, G.; Coruzzi, G.; Manarini, S.; Fruttero, R.; Gasco, A. *J. Med. Chem.* **2001**, *44*, 3463–3468.

(147) Visentin, S.; Amiel, P.; Fruttero, R.; Boschi, D.; Roussel, C.; Giusta, L.; Carbone, E.; Gasco, A. *J. Med. Chem.* **1999**, *42*, 1422–1427.

(148) Bertinaria, M.; Galli, U.; Sorba, G.; Fruttero, R.; Gasco, A.; Brenciaglia, M. I.; Scaltrito, M. M.; Dubini, F. *Drug. Dev. Res.* **2003**, *60*, 225–239.

(149) Bertinaria, M.; Sorba, G.; Medana, C.; Cena, C.; Adami, M.; Morini, G.; Pozzoli, C.; Coruzzi, G.; Gasco, A. *Helv. Chim. Acta* **2000**, *83*, 287–299.

(150) Tosco, P.; Bertinaria, M.; Stilo, A. Di; Cena, C.; Sorba, G.; Fruttero, R.; Gasco, A. *Bioorg. Med. Chem.* **2005**, *13*, 4750–4759.

(151) Moharram, S.; Zhou, A.; Wiebe, L. I.; Knaus, E. E. *J. Med. Chem.* **2004**, *47*, 1840–1846.

(152) Wang, T.; Zhang, Y.-H.; Kong, X.-W.; Lai, Y.-S.; Ji, H.; Chen, Y.-P.; Peng, S.-X. *Chem. Biodivers.* **2009**, *6*, 466–74.

(153) Min, T.; Yi, B.; Zhang, P.; Liu, J.; Zhang, C.; Zhou, H. *Med. Chem. Res.* **2009**, *18*, 495–510.

(154) Lai, Y.; Shen, L.; Zhang, Z.; Liu, W.; Zhang, Y.; Ji, H.; Tian, J. *Bioorg. Med. Chem. Lett.* **2010**, *20*, 6416–6420.

(155) Buonsanti, M. F.; Bertinaria, M.; Di Stilo, A.; Cena, C.; Fruttero, R.; Gasco, A. *J. Med. Chem.* **2007**, *50*, 5003–5011.

(156) Lolli, M. L.; Rolando, B.; Tosco, P.; Chaurasia, S.; Stilo, A. Di; Lazzarato, L.; Gorassini, E.; Ferracini, R.; Oliaro-Bosso, S.; Fruttero, R.; Gasco, A. *J. Med. Chem.* **2010**, *18*, 2428–2438.

(157) Schiefer, I. T.; VandeVrede, L.; Fa', M.; Arancio, O.; Thatcher, G. R. J. *J. Med. Chem.* **2012**, *55*, 3076–3087.

(158) Peng, S.; Wuu, J.; Mufson, E. J.; Fahnestock, M. *J. Neurochem.* **2005**, *93*, 1412–1421.

(159) Montanaro, G.; Bertinaria, M.; Rolando, B.; Fruttero, R.; Lucas, C. D.; Dorward, D. A.; Rossi, A. G.; Megson, I. L.; Gasco, A. *Bioorg. Med. Chem.* **2013**, *21*, 2107–2116.

(160) Brookes, P.; Walker, J. *J. Chem. Soc.* **1957**, 4409–4416.

(161) Fischer, E. *Justus Leibigs Ann. Chem.* **1882**, 316–339.

(162) Kato, H.; Hashimoto, H.; Ohta, M. *Nippon Kagaku Zasshi* **1957**, 707.

(163) Bartsch, H.; Montesano, R. *Carcinogenesis* **1984**, *5*, 1381–1393.

(164) Daeniker, H. U.; Druey, J. *Helv. Chim. Acta* **1962**, *45*, 2441–2462.

(165) Ruxer, J. M.; Mauger, J.; Bénard, D.; Lachoux, C. *J. Heterocycl. Chem.* **1995**, *32*, 643–654.

(166) Ohta, M.; Sato, S. *Bull. Chem. Soc. Jpn.* **1966**, *39*, 1269–1273.

(167) Parshin, V. A.; Olovyanishnikova, Z. A.; Yashunskii, V. G.; Mashkovskii, M. D. *Pharm. Chem. Journal.* **1981**, *15*, 333–337.

(168) Boehringer Sohm Ingelheim: Sydnonimine Derivatives **1969**, GB1219254.

(169) Götz, M.; Grozinger, K.; Oliver, J. T. *J. Med. Chem.* **1973**, *16*, 671–3.

(170) Bohn, H.; Schönafinger, K. *J. Cardiovascl. Pharmacol.* **1989**, *14*, Suppl 11 S6–12.

(171) Khmel'nitskaya, E.Y., Trukhacheva, L.A., Grigoriev, N.B., Kalinin, V.N., Cherepanov, I.A., Lebedev, S.N., and Granik, V. G. *Russ. Chem. Bull. Int. Ed.* **2004**, *53*, 2840–2844.

(172) Holm, P.; Kankaanranta, H.; Metsä-Ketelä, T.; Moilanen, E. *Eur. J. Pharmacol.* **1998**, *346*, 97–102.

(173) Chernov, V. A.; Yashunskii, V. G. *Dokl. Akad. Nauk SSSR* **1964**, *155*, 216.

(174) Oehme, P.; Gores, K.; K., S.; Petsch, G.; Faulhaber, H.; P., L. *Acta. Biol. Med. Ger.* **1965**, *14*, 369.

(175) Yashunskii, V. G.; Kholodov, L. E. *Russ. Chem. Rev.* **1980**, 28–45.

(176) Balakumaran, K.; Hugenholtz, P. G.; Tijssen, J. G. P.; Chadha, D. E. V. R. *Eur. Heart. J.* **1983**, *4*, 655–661.

(177) Bohn, H.; Beyerle, R.; Martorana, P. A.; Schönafinger, K. *J. Cardiovascl. Pharmacol.* **1991**, *18*.

(178) Soulère, L.; Bringaud, F.; Hoffmann, P. *J. Heterocycl. Chem.* **2003**, *40*, 943–947.

(179) Hagos, A.; Goddeeris, B. M.; Yilkal, K.; Alemu, T.; Fikru, R.; Yacob, H. T.; Feseha, G.; Claes, F. *Vet. Parasitol.* **2010**, *171*, 200–206.

(180) Cai, T. B.; Lu, D.; Tang, X.; Zhang, Y.; Landerholm, M.; Wang, P. G. *J. Org. Chem.* **2005**, *70*, 3518–3524.

(181) Tang, X.; Cai, T.; Wang, P. G. *Bioorg. Med. Chem. Lett.* **2003**, *13*, 1687–1690.

(182) Maples, K.; Sandstrom, T.; Su, Y.; Henderson, R. *Am. J. Respir. Cell. Mol. Biol.* **1992**, *4*, 538–543.

(183) Arroyo, C.; Kohno, M. *Free Radical Res. Commun.* **1991**, 145–155.

(184) Greenberg, S. S.; Wilcox, D. E.; Rubanyi, G. M. *Circ. Res.* **1990**, *67*, 1446–1452.

(185) Wang, Q.; Jacobs, J.; DeLeo, J.; Kruszyna, H.; Kruszyna, R.; Smith, R.; Wilcox, D. *Life Sci.* **1991**, *49*, PL55–PL60.

(186) Westernberger, U.; Thanner, S.; Ruf, H.; Gersonde, K.; Sutter, G.; Trentz, O. *Free Radical Res. Commun.* **1990**, *11*, 167–178.

(187) Kelm, M.; Feelisch, M.; Spahr, R.; Piper, H.-M.; Noack, E.; Schrader, J. *Biochem. Biophys. Res. Commun.* **1988**, *154*, 236–244.

(188) Tsikas, D. *J. Chromatogr. B.* **2007**, *851*, 51–70.

(189) Zafiriou, O. C.; McFarland, M. *Anal. Chem.* **1980**, *52*, 1662–1667.

(190) Menon, N.; Wolf, A.; Zehetgruber, M.; Bing, R. *Proc. Soc. Exp. Biol. Med.* **1989**, *191*, 316–319.

(191) Lee, C. H.; Akin-Olugbade, O.; Kirschenbaum, A. *Endocrin. Metab. Clin. North. Am.* **2011**, *40*, 565–575.

(192) Balk, S. P.; Ko, Y.-J.; Bubley, G. J. *J. Clin. Oncol.* **2003**, *21*, 383–391.

(193) Gnanapragasam, V. J.; Robson, C. N.; Leung, H. Y.; Neal, D. E. *BJU Int.* **2000**, *86*, 1001–1013.

(194) Mooradian, A. D.; Morley, J. E.; Korenman, S. G. *Endocr. Rev.* **1987**, *8*, 1–28.

(195) Thomas, L. N.; Douglas, R. C.; Rittmaster, R. S.; Too, C. K. L. *Prostate* **2009**, *69*, 595–602.

(196) Carter, B. S.; Beaty, T. H.; Steinberg, G. D.; Childs, B.; Walsh, P. C. *Proc. Natl. Acad. Sci. U.S.A.* **1992**, *89*, 3367–3371.

(197) Sakr, W. J.; Grignon, D. J.; Haas, G. P.; Heilbrun, L. K.; Pontes, J. E.; Crissman, J. D. *Cancer Risk Management Programme information for Primary Care, PSA testing for asymptomatic men*; 2008; p. NHS Cancer Screening Programmes: Sheffield.

(198) Huggins, C.; Hodges, C. V. *Cancer Res.* **1941**, *1*, 293–297.

(199) Hessels, D.; Schalken, J. A. *Asian J. Androl.* **2013**, *15*, 333–339.

(200) Wang, L.; Scherr, D.; Shariat, S. In *Prostate Cancer Diagnosis SE-7*; Jones, J. S., Ed.; Humana Press, 2013; pp. 73–84.

(201) Quinn, D.; Swanson, G. In *Biomarkers in Oncology SE - 9*; Lenz, H.-J., Ed.; Springer New York, 2013; pp. 189–247.

(202) Schröder, F. H.; Hermanek, P.; Denis, L.; Fair, W. R.; Gospodarowicz, M. K.; Pavone-Macaluso, M. *Prostate* **1992**, *21*, 129–138.

(203) Barry, M. J. *New Eng. J. Med.* **2001**, *344*, 1373–1377.

(204) Carlin, B. I.; Andriole, G. L. *Cancer* **2000**, *88*, 2989–1994.

(205) Bubendorf, L.; Schöpfer, A.; Wagner, U.; Sauter, G.; Moch, H.; Willi, N.; Gasser, T. C.; Mihatsch, M. J. *Hum. Pathol.* **2000**, *31*, 578–583.

(206) Steinberg, G. D.; Carter, B. S.; Beaty, T. H.; Childs, B.; Walsh, P. C. *Prostate* **1990**, *17*, 337–347.

(207) Smith, J. R.; Freije, D.; Carpten, J. D.; Grönberg, H.; Xu, J.; Isaacs, S. D.; Brownstein, M. J.; Bova, G. S.; Guo, H.; Bujnovszky, P.; Nusskern, D. R.; Damber, J.-E.; Bergh, A.; Emanuelsson, M.; Kallioniemi, O. P.; Walker-Daniels, J.; Bailey-Wilson, J. E.; Beaty, T. H.; Meyers, D. A.; Walsh, P. C.; Collins, F. S.; Trent, J. M.; Isaacs, W. B. *Science* **1996**, *274* , 1371–1374.

(208) McIndoe, R. A.; Stanford, J. L.; Gibbs, M.; Jarvik, G. P.; Brandzel, S.; Neal, C. L.; Li, S.; Gammack, J. T.; Gay, A. A.; Goode, E. L.; Hood, L.; Ostrander, E. A. *Am. J. Hum. Genet.* **1997**, *61*, 347–353.

(209) Jarvik, G. P.; Stanford, J. L.; Goode, E. L.; McIndoe, R.; Kolb, S.; Gibbs, M.; Hood, L.; Ostrander, E. A. *J. Natl. Cancer Inst. Monogr.* **1999**, *1999*, 81–87.

(210) Cooney, K. A.; Huang, L.; Sandler, H. M.; Lange, E.; Lange, K.; Miesfeldt, S.; McCarthy, J. D.; Montie, J. E.; Oesterling, J. E. *J. Natl. Cancer Inst.* **1997**, *89*, 955–959.

(211) Grönberg, H.; Smith, J.; Emanuelsson, M.; Jonsson, B.-A.; Bergh, A.; Carpten, J.; Isaacs, W.; Xu, J.; Meyers, D.; Trent, J.; Damber, J.-E. *Am. J. Hum. Genet.* **1999**, *65*, 134–140.

(212) Gurel, B.; Alexander, R.; Cheng, L.; Marzo, A. In *Molecular Surgical Pathology SE - 10*; Cheng, L.; Eble, J. N., Eds.; Springer New York, 2013; pp. 213–228.

(213) Peehl, D. M. *Cancer* **1993**, *71*, 1159–1164.

(214) Castro, E.; Goh, C.; Olmos, D.; Saunders, E.; Leongamornlert, D.; Tymrakiewicz, M.; Mahmud, N.; Dadaev, T.; Govindasami, K.; Guy, M.; Sawyer, E.; Wilkinson, R.; Ardern-Jones, A.; Ellis, S.; Frost, D.; Peock, S.; Evans, D. G.; Tischkowitz, M.; Cole, T.; Davidson, R.; Eccles, D.; Brewer, C.; Douglas, F.; Porteous, M. E.; Donaldson, A.; Dorkins, H.; Izatt, L.; Cook, J.; Hodgson, S.; Kennedy, M. J.; Side, L. E.; Eason, J.; Murray, A.; Antoniou, A. C.; Easton, D. F.; Kote-Jarai, Z.; Eeles, R. *J. Clin. Oncol.* **2013**.

(215) Miki, Y.; Swensen, J.; Shattuck-Eidens, D.; Futreal, P. A.; Harshman, K.; Tavtigian, S.; Liu, Q.; Cochran, C.; Bennett, L. M.; Ding, W.; et, al. *Science* **1994**, *266* , 66–71.

(216) King, M.-C.; Marks, J. H.; Mandell, J. B.; Group, T. N. Y. B. C. S. *Science* **2003**, *302*, 643–646.

(217) Karayi, M. K.; Neal, D. E.; Markham, A. F. *BJU Int.* **2000**, *86*, 659–669.

(218) Heinlein, C. A.; Chang, C. *Endocr. Rev.* **2004**, *25*, 276–308.

(219) Pereira de Jésus-Tran, K.; Côté, P.-L.; Cantin, L.; Blanchet, J.; Labrie, F.; Breton, R. *Protein Sci.* **2006**, *15*, 987–999.

(220) Brinkmann, A. O.; Faber, P. W.; Van Rooij, H. C. J.; Kuiper, G. G. J. M.; Ris, C.; Klaassen, P.; Van der Korput, J. A. G. M.; Voorhorst, M. M.; Van Laar, J. H.; Mulder, E.; Trapman, J. *J. Steroid Biochem. Mol. Biol.* **1989**, *34*, 307–310.

(221) Smith, D. F.; Toft, D. O. *Mol. Endocrinol.* **2008**, *22*, 2229–2240.

(222) Clark, P. A.; Rogol, A. D. *Endocrin. Metab. Clin. North. Am.* **1996**, *25*, 665–681.

(223) De Marzo, A. M.; Nelson, W. F.; Meeker, A.; Coffey, D. S. *J. Urol.* **1998**, *160*, 2381–2392.

(224) Sadi, M. V; Walsh, P. C.; Barrack, E. R. *Cancer* **1991**, *67*, 3057–3064.

(225) English, H. F.; Kyprianou, N.; Isaacs, J. T. *Prostate* **1989**, *15*, 233–250.

(226) Ruizeveld De Winter, J. A.; Janssen, P. J.; Sleddens, H. M.; Verleun-Mooijman, M. C.; Trapman, J.; Brinkmann, A. O.; Santerse, A. B.; Schröder, F. H.; Van Der Kwast, T. H. *Am. J. Pathol.* **1994**, *144*, 735–746.

(227) Zegarra-Moro, O. L.; Schmidt, L. J.; Huang, H.; Tindall, D. J. *Cancer Res.* **2002**, *62*, 1008–1013.

(228) Feldman, B. J.; Feldman, D. *Nat. Rev. Cancer* **2001**, *1*, 34–45.

(229) Linja, M. J.; Savinainen, K. J.; Saramäki, O. R.; Tammela, T. L. J.; Vessella, R. L.; Visakorpi, T. *Cancer Res.* **2001**, *61*, 3550–3555.

(230) Visakorpi, T.; Hyytinen, E.; Koivisto, P.; Tanner, M.; Keinanen, R.; Palmberg, C.; Palotie, A.; Tammela, T.; Isola, J.; Kallioniemi, O.-P. *Nat. Genet.* **1995**, *9*, 401–406.

(231) Cher, M. L.; Bova, G. S.; Moore, D. H.; Small, E. J.; Carroll, P. R.; Pin, S. S.; Epstein, J. I.; Isaacs, W. B.; Jensen, R. H. *Cancer Res.* **1996**, *56*, 3091–3102.

(232) Zong, Y.; Goldstein, A. S. *Nat. Rev. Urol.* **2013**, *10*, 90–98.

(233) Labrie, A.; Dupont, A.; Bélanger, A.; St-Arnaud, R.; Giguère, M.; Lacourcière, Y.; Emond, J.; Monfette, G. *Endocr. Rev.* **1986**, *7*, 67–74.

(234) Tan, J.; Sharief, Y.; Hamil, K. G.; Gregory, C. W.; Zang, D.-Y.; Sar, M.; Gumerlock, P. H.; deVere White, R. W.; Pretlow, T. G.; Harris, S. E.; Wilson, E. M.; Mohler, J. L.; French, F. S. *Mol. Endocrinol.* **1997**, *11*, 450–459.

(235) Veldscholte, J.; Voorhorst-Ogink, M. M.; Bolt-de Vries, J.; Van Rooij, H. C. J.; Trapman, J.; Mulder, E. *BBA- Mol. Cell. Res.* **1990**, *1052*, 187–194.

(236) Culig, Z.; Hobisch, A.; Cronauer, M. V; Cato, A. C.; Hittmair, A.; Radmayr, C.; Eberle, J.; Bartsch, G.; Klocker, H. *Mol. Endocrinol.* **1993**, *7*, 1541–1550.

(237) Miyamoto, H.; Yeh, S.; Wilding, G.; Chang, C. *Proc. Natl. Acad. Sci. U.S.A.* **1998**, *95*, 7379–7384.

(238) Terekawa, T.; Miyake, H.; Kumano, M.; Sakai, I.; Fujisawa, M. *Oncol. Rep.* **2010**, *25*, 1395–1399.

(239) Jenster, G. *J. Pathol.* **2000**, *191*, 227–228.

(240) Shi, X.-B.; Ma, A.-H.; Xia, L.; Kung, H.-J.; De Vere White, R. W. *Cancer Res.* **2002**, *62*, 1496–1502.

(241) Yeh, S.; Lin, H.-K.; Kang, H.-Y.; Thin, T. H.; Lin, M.-F.; Chang, C. *Proc. Natl. Acad. Sci. U.S.A.* **1999**, *96*, 5458–5463.

(242) Shukla, S.; MacLennan, G. T.; Hartman, D. J.; Fu, P.; Resnick, M. I.; Gupta, S. *Int. J. Cancer* **2007**, *121*, 1424–1432.

(243) Bélanger, A.; Van Halbeek, H.; Graves, H. C. B.; Grandbois, K.; Stamey, T. A.; Huang, L.; Poppe, I.; Labrie, F. *Prostate* **1995**, *27*, 187–197.

(244) Stenman, U.-H.; Leinonen, J.; Zhang, W.-M.; Finne, P. *Semin. Cancer. Biol.* **1999**, *9*, 83–93.

(245) Moyer, V. A. *Ann. Intern. Med.* **2012**, *157*, 120–134.

(246) Folkman, J. *Nat. Med. Med.* **1995**, *1*, 27–31.

(247) Cleutjens, K. B. J. M.; Van Eekelen, C. C. E. M.; Van der Korput, H. A. G. M.; Brinkmann, A. O.; Trapman, J. *J. Biol. Chem.* **1996**, *271*, 6379–6388.

(248) Bostwick, D. G.; Pacelli, A.; Blute, M.; Roche, P.; Murphy, G. P. *Cancer* **1998**, *82*, 2256–2261.

(249) Kawakami, M.; Nakayama, J. *Cancer Res.* **1997**, *57*, 2321–2324.

(250) Silver, D. A.; Pellicer, I.; Fair, W. R.; Heston, W. D.; Cordon-Cardo, C. *Clin. Cancer Res.* **1997**, *3*, 81–85.

(251) Davis, M. I.; Bennett, M. J.; Thomas, L. M.; Bjorkman, P. J. *Proc. Natl. Acad. Sci. U.S.A.* **2005**, *102*, 5981–5986.

(252) Pinto, J. T.; Suffoletto, B. P.; Berzin, T. M.; Qiao, C. H.; Lin, S.; Tong, W. P.; May, F.; Mukherjee, B.; Heston, W. D. *Clin. Cancer Res.* **1996**, *2*, 1445–1451.

(253) Liu, H.; Rajasekaran, A. K.; Moy, P.; Xia, Y.; Kim, S.; Navarro, V.; Rahmati, R.; Bander, N. H. *Cancer Res.* **1998**, *58*, 4055–4060.

(254) Murphy, G. P.; Elgamal, A. A.; Su, S. L.; Bostwick, D. G.; Holmes, E. H. *Cancer* **1998**, *83*, 2259–2269.

(255) Smith-Jones, P. M.; Vallabahajosula, S.; Goldsmith, S. J.; Navarro, V.; Hunter, C. J.; Bastidas, D.; Bander, N. H. *Cancer Res.* **2000**, *60*, 5237.

(256) Kozikowski, A. P.; Nan, F.; Conti, P.; Zhang, J.; Ramadan, E.; Bzdega, T.; Wroblewska, B.; Neale, J. H.; Pshenichkin, S.; Wroblewski, J. T. *J. Med. Chem.* **2001**, *44*, 298–301.

(257) Maresca, K. P.; Hillier, S. M.; Femia, F. J.; Keith, D.; Barone, C.; Joyal, J. L.; Zimmerman, C. N.; Kozikowski, A. P.; Barrett, J. A.; Eckelman, W. C.; Babich, J. W. *J. Med. Chem.* **2008**, *52*, 347–357.

(258) Fleming, C.; Wasson, J. H.; Albertsen, P. C. *JAMA* **1993**, *269*, 2650–2658.

(259) Young, H. H. *J. Urol.* **1905**, *168*, 914–21.

(260) Millin, T. *J. Urol.* **1948**, *59*, 267–280.

(261) Walsh, P. C.; Partin, A. W.; Epstein, J. I. *J. Urol.* **1994**, *152*, 1831–1836.

(262) Liss, M. A.; Lusch, A.; Morales, B.; Beheshti, N.; Skarecky, D.; Narula, N.; Osann, K.; Ahlering, T. E. *J. Urol.* **2012**, *188*, 2205–2211.

(263) Eisenberger, M. A.; Blumenstein, B. A.; Crawford, E. D.; Miller, G.; McLeod, D. G.; Loehrer, P. J.; Wilding, G.; Sears, K.; Culkin, D. J.; Thompson, I. M.; Bueschen, A. J.; Lowe, B. A. *New Eng. J. Med.* **1998**, *339*, 1036–1042.

(264) Pollack, A.; Smith, L. G.; Von Eschenbach, A. C. *Int. J. Radiat. Oncol.* **2000**, *48*, 507–512.

(265) Vicini M.D., F. A.; Horwitz M.D., E. M.; Kini M.D., V. R.; Stromberg M.D., J. S.; Martinez M.D. Alvaro A, F. *Int. J. Radiat. Oncol. Biol. Phys.* **1998**, *40*, 1101–1110.

(266) Zelefsky, M. J.; Fuks, Z.; Hunt, M.; Lee, H. J.; Lombardi, D.; Ling, C. C.; Reuter, V. E.; Venkatraman, E. S.; Leibel, S. A. *J. Urol.* **2001**, *166*, 876–881.

(267) Wallner, K. *Oncology* **1991**, *5*, 115–122.

(268) Blasko, J. C.; Grimm, P. D.; Sylvester, J. E.; Badiozamani, K. R.; Hoak, D.; Cavanagh, W. *Int. J. Radiat. Oncol. Biol. Phys.* **2000**, *46*, 839–850.

(269) Murphy, M. K.; Piper, R. K.; Greenwood, L. R.; Mitch, M. G.; Lamperti, P. J.; Seltzer, S. M.; Bales, M. J.; Phillips, M. H. *Med. Phys.* **2004**, *31*, 1529–1538.

(270) Chin, Y. S.; Bullard, J.; Bryant, L.; Bownes, P.; Ostler, P.; Hoskin, P. J. *Clin. Oncol.* **2006**, *18*, 474–479.

(271) Parker, C.; Heinrich, D.; O'Sullivan, J. M.; Fossa, S.; Chodacki, A.; Demkow, T.; Cross, A.; Bolstad, B.; Garcia-Vargas, J.; Sartor, O. *Eur. J. Cancer* **2011**, *47*, 3.

(272) Hafeez, S.; Parker, C. *Exp. Opin. Exp. Drug* **2013**, *22*, 379–387.

(273) McLeod, D. G.; Crawford, E. D.; DeAntoni, E. P. *Eur. Urol.* **1997**, *32 Suppl 3*, 70–77.

(274) Borgmann, V.; Hardt, W.; Schmidt-Gollwitzer, M.; Adenauer, H.; Nagel, R. *Lancet* **1982**, *319*, 1097–1099.

(275) Karten, M. J.; Rivier, J. E. *Endocr. Rev.* **1986**, *7*, 44–66.

(276) Thompson, I. M. *Rev. Urol.* **2001**, *Suppl. 3*, S10–S14.

(277) Labrie, F. *Cancer* **1993**, *72*, 3816–3827.

(278) Neumann, F. *J. Steroid Biochem. Mol. Biol.id* **1983**, *19*, 391–402.

(279) Rabe, T.; Feldmann, K.; Heinemann, L.; Runnebaum, B. *Drug Safety* **1996**, *14*, 25–38.

(280) Holdaway, I. M.; Croxson, M. S.; Evans, M. C.; France, J.; Sheehan, A.; Wilson, T.; Ibbertson, H. K. *Acta Endocrinol.-COP* **1983**, *104*, 222–226.

(281) Tyrrell, C. J.; Kaisary, A. V; Iversen, P.; Anderson, J. B.; Baert, L.; Tammela, T.; Chamberlain, M.; Webster, A.; Blackledge, G. *Eur. Urol.* **1998**, *33*, 447–456.

(282) Katchen, B.; Buxbaum, S. *J. Clin. Endocrinol. Metab.* **1975**, *41*, 373–379.

(283) Kassouf, W.; Tanguay, S.; Aprikian, A. G. *J. Urol.* **2003**, *169*, 1742–1744.

(284) Tucker, H.; Crook, J. W.; Chesterson, G. J. *J. Med. Chem.* **1988**, *31*, 954–959.

(285) Yin, D.; He, Y.; Perera, M. A.; Hong, S. S.; Marhefka, C.; Stourman, N.; Kirkovsky, L.; Miller, D. D.; Dalton, J. T. *Mol. Pharmacol.* **2003**, *63*, 211–223.

(286) Cook, T.; Sheridan, W. P. *The Oncologist* **2000**, *5* , 162–168.

(287) Klotz, L.; Boccon-Gibod, L.; Shore, N. D.; Andreou, C.; Persson, B.-E.; Cantor, P.; Jensen, J.-K.; Olesen, T. K.; Schröder, F. H. *BJU Int.* **2008**, *102*, 1531–1538.

(288) Reid, A. H. M.; Attard, G.; Barrie, E.; De Bono, J. S. *Nat. Clin. Pract. Oncol.* **2008**, *5*, 610–620.

(289) Van Wauwe, J. P.; Janssen, P. A. J. *J. Med. Chem.* **1989**, *32*, 2231–2239.

(290) Stanbrough, M.; Bubley, G. J.; Ross, K.; Golub, T. R.; Rubin, M. A.; Penning, T. M.; Febbo, P. G.; Balk, S. P. *Cancer Res.* **2006**, *66*, 2815–2825.

(291) Potter, G. A.; Barrie, S. E.; Jarman, M.; Rowlands, M. G. *J. Med. Chem.* **1995**, *38*, 2463–2471.

(292) Denis, L. J.; Griffiths, K. *Semin. Surg. Oncol.* **2000**, *18*, 52–74.

(293) Forti, G.; Salerno, R.; Moneti, G.; Zoppi, S.; Fiorelli, G.; Marinoni, T.; Natali, A.; Costantini, A.; Serio, M.; Martini, L.; Motta, M. *JCEM* **1989**, *68*, 461–468.

(294) Russell, P. J.; Bennett, S.; Stricker, P. *Clin. Chem.* **1998**, *44*, 705–723.

(295) Buttyan, R.; Ghafar, M. A.; Shabsigh, A. *Curr. Opin. Urol.* **2000**, *10*.

(296) Gilligan, T.; Kantoff, P. W. *Urology* **2002**, *60*, 94–100.

(297) Petrylak, D. P.; Tangen, C. M.; Hussain, M. H. A.; Lara, P. N.; Jones, J. A.; Taplin, M. E.; Burch, P. A.; Berry, D.; Moinpour, C.; Kohli, M.; Benson, M. C.; Small, E. J.; Raghavan, D.; Crawford, E. D. *New Eng. J. Med.* **2004**, *351*, 1513–1520.

(298) Stewart, G. D.; Nanda, J.; Brown, D. J. G.; Riddick, A. C. P.; Ross, J. A.; Habib, F. K. *Int. J. Cancer* **2009**, *124*, 223–32.

(299) Kaighn, M. E.; Narayan, K. S.; Ohnuki, Y.; Lechner, J. F.; W., J. L. *Invest. Urol.* **1979**, *17*, 16–23.

(300) Pulukuri, S. M.; Gondi, C. S.; Lakka, S. S.; Jutla, A.; Estes, N.; Gujrati, M.; Rao, J. S. *J. Biol. Chem.* **2005**, *280*, 36529–36540.

(301) Sun, H.-L.; Liu, Y.-N.; Huang, Y.-T.; Pan, S.-L.; Huang, D.-Y.; Guh, J.-H.; Lee, F.-Y.; Kuo, S.-C.; Teng, C.-M. *Oncogene* **2007**, *26*, 3941–3951.

(302) Zhong, H.; Agani, F.; Baccala, A. A.; Laughner, E.; Rioseco-Camacho, N.; Isaacs, W. B.; Simons, J. W.; Semenza, G. L. *Cancer Res.* **1998**, *58*, 5280–5284.

(303) Zundel, W.; Schindler, C.; Haas-Kogan, D.; Koong, A.; Kaper, F.; Chen, E.; Gottschalk, A. R.; Ryan, H. E.; Johnson, R. S.; Jefferson, A. B.; Stokoe, D.; Giaccia, A. J. *Genes. Dev.* **2000**, *14*, 391–396.

(304) Stewart, G. D.; Nanda, J.; Katz, E.; Bowman, K. J.; Christie, J. G.; Brown, D. J. G.; McLaren, D. B.; Riddick, A. C. P.; Ross, J. A.; Jones, G. D. D.; Habib, F. K. *Biochem. Pharmacol.* **2011**, *81*, 203–210.

(305) Royle, J. S.; Ross, J. A.; Ansell, I. A. N.; Bollina, P.; Tulloch, D. N.; K., H. F. *J. Urol.* **2004**, *172*, 338–344.

(306) Goluboff, E. T.; Shabsigh, A.; Saidi, J. A.; Weinstein, I. B.; Mitra, N.; Heitjan, D.; Piazza, G. A.; Pamukcu, R.; Buttyan, R.; Olsson, C. A. *Urology* **1999**, *53*, 440–445.

(307) Sinicrope, F. A.; Penington, R. C. *Mol. Cancer. Ther.* **2005**, *4*, 1475–1483.

(308) Fogli, S.; Banti, I.; Stefanelli, F.; Picchianti, L.; Digiacomo, M.; Macchia, M.; Breschi, M. C.; Lapucci, A. *Eur. J. Med. Chem.* **2010**, *45*, 5100–5107.

(309) Kikuchi, S.; Konishi, H.; Hashimoto, Y. *Tetrahedron* **2005**, *61*, 3587–3591.

(310) 5 g of ca. 3 mmol/g at a cost of £130.00 (Sigma Aldrich, 93093-5G, June 2013).

(311) Trost, B. M. *Strategy and Efficienty in Modern Chemistry: Reduction v. 8*; Pergamon, 1992; pp. 404–407.

(312) Neises, B.; Steglich, W. *Angew. Chem. Int. Ed.* **1978**, *17*, 522–524.

(313) Keck, G. E.; Heumann, S. A. *Org. Lett.* **2008**, *10*, 4783–4786.

(314) Treves, K.; Shragina, L.; Rudich, Y. *J. Phys. Chem. A* **2002**, *106*, 5902–5907.

(315) Nortcliffe, A. Towards N-diazeniumdiolate analogues of Sulindac, MSc Thesis, University of Edinburgh, 2010.

(316) Taillier, C.; Gille, B.; Bellosta, V.; Cossy, J. *J. Org. Chem.* **2005**, *70*, 2097–2108.

(317) Greene, T. W.; Wuts, P. G. M. *Protective Groups in Organic Synthesis*; 3rd ed.; John Wiley & Sons, Inc., 1991.

(318) Biava, M.; Porretta, G. C.; Poce, G.; Battilocchio, C.; Alfonso, S.; Rovini, M.; Valenti, S.; Giorgi, G.; Calderone, V.; Martelli, A.; Testai, L.; Sautebin, L.; Rossi, A.; Papa, G.; Ghelardini, C.; Di Cesare Mannelli, L.; Giordani, A.; Anzellotti, P.; Bruno, A.; Patrignani, P.; Anzini, M. *J. Med. Chem.* **2011**, *54*, 7759–7771.

(319) Shan, R.; Velazquez, C.; Knaus, E. E. *J. Med. Chem.* **2003**, *47*, 254–261.

(320) Mayants, A. G.; Pyreseva, K. G.; Gordeichuk, S. S. *Zhurnal Organicheskoi Khimii* **1986**, *22*, 2120–2123.

(321) Farrar, W. V *J. Chem. Soc.* **1964**, 904.

(322) Nitromed 2007, p. WO2007/59311 A2 p62–63.

(323) Bahrami, K.; Khodaei, M. M.; Sheikh Arabi, M. *J. Org. Chem.* **2010**, *75*, 6208–6213.

(324) Lautens, M.; Paquin, J.-F.; Piguel, S. *J. Org. Chem.* **2002**, *67*, 3972–3974.

(325) Woo, Y.-H.; Fernandes, R. P. M.; Proteau, J. P. *Bioorg. Med. Chem.* **2006**, *14*, 2375–2385.

(326) Leclerc, E.; Vrancken, E.; Mangeney, P. *J. Org. Chem.* **2002**, *67*, 8928–37.

(327) Halder, S.; Satyam, A. *Tetrahedron Lett.* **2001**, *52*, 1179–1182.

(328) Stefaniak, L.; Witanowski, M.; Kamienski, B.; Webb, G. A. *Org. Mag. Res.* **1980**, *13*, 274–276.

(329) Sun, C.; Zhu, J.; Wang, H.; Jin, J.; Yang, D.; Xing, J. *Eur. J. Med. Chem.* **2011**, *46*, 11–20.

(330) Schröder, F.; Crawford, E. D.; Axcrona, K.; Payne, H.; Keane, T. E. *BJU Int.* **2012**, *109*, 1–12.

(331) Seruga, B.; Ocana, A.; Tannock, I. F. *Nat. Rev. Clin. Oncol.* **2011**, *8*, 12–23.

(332) Reid, A. H. M.; Attard, G.; Barrie, E.; Bono, J. S. De *Nat. Clin. Pract. Oncol.* **2008**, *5*, 610–620.

(333) Small, E. J.; Halabi, S.; Dawson, N. A.; Stadler, W. M.; Rini, B. I.; Picus, J.; Gable, P.; Torti, F. M.; Kaplan, E.; Vogelzang, N. J. *J. Clin. Oncol.* **2004**, *22*, 1025–1033.

(334) McCague, R.; Rowlands, M. G.; Barrie, S. E.; Houghton, J. *J. Med. Chem.* **1990**, *33*, 3050–3055.

(335) Laughton, C. A.; Neidle, S. *J. Med. Chem.* **1990**, *33*, 3055–3060.

(336) Mak, A. Y.; Swinney, D. C. *J. Am. Chem. Soc.* **1992**, *114*, 8309–8310.

(337) Hosaka, M.; Oshima, H.; Troen, P. *Acta Endocrinol.* **1980**, *94*, 389–396.

(338) De Bono, J. S.; Logothetis, C. J.; Molina, A.; Fizazi, K.; North, S.; Chu, L.; Chi, K. N.; Jones, R. J.; Goodman, O. B.; Saad, F.; Staffurth, J. N.; Mainwaring, P.; Harland, S.; Flaig, T. W.; Hutson, T. E.; Cheng, T.; Patterson, H.; Hainsworth, J. D.; Ryan, C. J.; Sternberg, C. N.; Ellard, S. L.; Fléchon, A.; Saleh, M.; Scholz, M.; Efstathiou, E.; Zivi, A.; Bianchini, D.; Loriot, Y.; Chieffo, N.; Kheoh, T.; Haqq, C. M.; Scher, H. I. *New Eng. J. Med.* **2011**, *364*, 1995–2005.

(339) Habib, F. K. University of Edinburgh, Personal Communication.

(340) Handratta, V. D.; Vasaitis, T. S.; Njar, V. C. O.; Gediya, L. K.; Kataria, R.; Chopra, P.; Newman, D.; Farquhar, R.; Guo, Z.; Qiu, Y.; Brodie, A. M. H. *J. Med. Chem.* **2005**, *48*, 2972–2984.

(341) Sarma, J. C. *Ind. J. Chem. Section B.* **1994**, *33*, 790–791.

(342) De Nanteuil, G.; Portevin, B.; Gloanec, P.; Parmentier, J.-G.; Benoist, A.; Verbeuren, T.; Rupin, A.; Courchay, C.; Simonet, S. US2009082393A1.pdf **2009**, US 2009/0082393 A1.

(343) Tanrikulu, Y.; Krüger, B.; Proschak, E. *Drug Discovery Today* **2013**, *18*, 358–364.

(344) Murray, C. W.; Rees, D. C. *Nat. Chem.* **2009**, *1*, 187–192.

(345) Love, R. R.; Leventhal, H.; Easterling, D. V; Nerenz, D. R. *Cancer* **1989**, *63*, 604–612.

(346) Lesch, H. P.; Kaikkonen, M. U.; Pikkarainen, J. T.; Ylä-Herttuala, S. *Exp. Opin. Drug. Del.* **2010**, *7*, 551–564.

(347) Zhao, X.; Li, H.; Lee, R. J. *Exp. Opin. Drug. Del.* **2008**, *5*, 309–319.

(348) Huang, C.-M.; Wu, Y.-T.; Chen, S.-T. *Chemistry & Biology* **2000**, *7*, 453–461.

(349) Ruoslahti, E. *Annual Review of Cell and Developmental Biology* **1996**, *12*, 697–715.

(350) Rojanasakul, Y. *Adv. Drug Deliv. Rev.* **1996**, *18*, 115–131.

(351) Shin, S. U.; Friden, P.; Moran, M.; Olson, T.; Kang, Y. S.; Pardridge, W. M.; Morrison, S. L. *Proc. Natl. Acad. Sci. U.S.A.* **1995**, *92*, 2820–2824.

(352) Ting, R.; Harwig, C.; auf dem Keblller, U.; McCormick, S.; Austin, P.; Overall, C. M.; Adam, M. J.; Ruth, T. J.; Perrin, D. M. *J. Am. Chem. Soc.* **2008**, *130*, 12045–12055.

(353) Dal Pozzo, A.; Ni, M.-H.; Esposito, E.; Dallavalle, S.; Musso, L.; Bargiotti, A.; Pisano, C.; Vesci, L.; Bucci, F.; Castorina, M.; Foderà, R.; Giannini, G.; Aulicino, C.; Penco, S. *Bioorg. Med. Chem.* **2010**, *18*, 64–72.

(354) Pierschbacher, M. D.; Ruoslahti, E. *Nature* **1984**, *309*, 30–33.

(355) Gumbiner, B. M. *Cell* **1996**, *84*, 345–357.

(356) Danhier, F.; Breton, A. Le; Préat, V. *Mol. Pharmaceut.* **2012**, *9*, 2961–2973.

(357) Pierschbacher, M. D.; Polarek, J. W.; Craig, W. S.; Tschopp, J. F.; Sipes, N. J.; Harper, J. R. *J. Cell. Biochem.* **1994**, *56*, 150–154.

(358) Desgrosellier, J. S.; Cheresh, D. A. *Nat. Rev. Cancer* **2010**, *10*, 9–22.

(359) Takayama, S.; Ishii, S.; Ikeda, T.; Masamura, S.; Doi, M.; Kitajima, M. *Anticancer Res.* **2005**, *25*, 79.

(360) Furger, K. A.; Allan, A. L.; Wilson, S. M.; Hota, C.; Vantyghem, S. A.; Postenka, C. O.; Al-Katib, W.; Chambers, A. F.; Tuck, A. B. *Mol. Cancer Res.* **2003**, *1*, 810.

(361) Sheldrake, H. M.; Patterson, L. H. *Curr. Cancer Drug Targets* **2009**, *9*, 519.

(362) Hosotani, R.; Kawaguchi, M.; Masui, T.; Koshiba, T.; Ida, J.; Fujimoto, K.; Wada, M.; Doi, R.; Imamura, M. *Pancreas* **2002**, *25*, e30–35.

(363) Humphries, M. J.; Akiyama, S. K.; Komoriya, A.; Olden, K.; M., Y. K. *J. Cell. Biol.* **1986**, *103*, 2637–2647.

(364) Saiki, I.; J., M.; Matsuno, K.; Ogawa, R.; Nishi, N.; Tokura, S.; Azuma, I. *Jpn. J. Cancer. Res.* **1990**, *81*, 660–667.

(365) Gehlsen, K. R.; Argraves, W. S.; Pierschbacher, M. D.; Ruoslahti, E. *J. Cell. Biol.* **1988**, *106*, 925–930.

(366) Cheng, Z.; Levi, J.; Xiong, Z.; Gheysens, O.; Keren, S.; Chen, X.; Gambhir, S. S. *Bioconjug. Chem.* **2006**, *17*, 662–669.

(367) Dall'Angelo, S.; Zhang, Q.; Fleming, I. N.; Piras, M.; Schweiger, L. F.; O'Hagan, D.; Zanda, M. *Org. Biomol. Chem.* **2013**, *11*, 4551–4558.

(368) Mukhopadhyay, S.; Barnés, C. M.; Haskel, A.; Short, S. M.; Barnes, K. R.; Lippard, S. J. *Bioconjug. Chem.* **2007**, *19*, 39–49.

(369) Hertzberg, R. P.; Caranfa, M. J.; Hecht, S. M. *Biochemistry* **1989**, *28*, 4629–4638.

(370) Richter, J. M.; Whitefield, B. W.; Maimone, T. J.; Lin, D. W.; Castroviejo, M. P.; Baran, P. S. *J. Am. Chem. Soc.* **2007**, *129*, 12857–12869.

(371) Lassoie, M.-A.; Broeders, F.; Collart, P.; Defrère, L.; De Laveleye-Defais, F.; Demaude, T.; Gassama, A.; Guillaumet, G.; Hayez, J.-C.; Kiss, L.; Knerr, L.; Nicolas, J.-M.; Norsikian, S.; Quéré, L.; Routier, S.; Verbois, V.; Provins, L. *Bioorg. Med. Chem. Lett.* **2007**, *17*, 142–146.

(372) Fruttero, R.; Crosetti, M.; Chegaev, K.; Guglielmo, S.; Gasco, A.; Berardi, F.; Niso, M.; Perrone, R.; Panaro, M. A.; Colabufo, N. A. *J. Med. Chem.* **2010**, *53*, 5467–5475.

(373) Torrini, I.; Zecchini, P. Z.; Agrosi, F.; Paradisi, M. P. *J. Heterocycl. Chem.* **1986**, *23*, 1459–1463.

(374) Wang, A. X.; Zheng, B. Z.; D'Andrea, S.; Meanwell, Nicholas, A.; Scola, P. M. **2008**, WO2008064057 (A1).

(375) Gurevich, V. S.; Lominadze, D. G.; Adeagbo, A. S. O.; Burov, S. V; Popov, Y. G.; Leko, M. V; Miller, F. N.; Schuschke, D. A. *Pharmacol.* **1997**, *55*, 1–9.

(376) Welsh, D. J.; Smith, D. K. *Org. Biomol. Chem.* **2011**, *9*, 4795–4801.

(377) Allen, D. R.; Kyung-Lee, S.-H. Preparation of protected amino acids **2003**, US2003/235430 A1.

(378) Deal, K. A.; Welch, M. J. *J. Med. Chem.* **1997**, *40*, 3986–3989.

(379) Carpino, L. A.; Sadat-Aalaee, D.; Chao, H. G.; DeSelms, R. H. *J. Am. Chem. Soc.* **1990**, *112*, 1515–1521.

(380) Carpino, L. A.; El-Faham, A. *Tetrahedron* **1999**, *55*, 6813–6830.

(381) Marin, J.; Briand, J.-P.; Guichard, G. *Eur. J. Org. Chem.* **2008**, *2008*, 1005–1012.

(382) Machauer, R.; Waldmann, H. *Angew. Chem. Int. Ed.* **2000**, *39*, 1449–1453.

(383) Wen, S.; Packham, G.; Ganesan, A. *J. Org. Chem.* **2008**, *73*, 9353–9361.

(384) Dijkgraaf, I.; Rijnders, A. Y.; Soede, A.; Dechesne, A. C.; Van Esse, G. W.; Brouwer, A. J.; Corstens, F. H. M.; Boerman, O. C.; Rijkers, D. T. S.; Liskamp, R. M. J. *Org. Biomol. Chem.* **2007**, *5*, 935–944.

(385) Haubner, R.; Gratias, R.; Diefenbach, B.; Goodman, S. L.; Jonczyk, A.; Kessler, H. *J. Am. Chem. Soc.* **1996**, *118*, 7461–7472.

(386) Nortcliffe, A.; Botting, N. P.; O'Hagan, D. *Org. Biomol. Chem.* **2013**, *11*, 4657–4671.

(387) Nagib, D. A.; MacMillan, D. W. C. *Nature* **2011**, *480*, 224–228.

(388) Ji, Y.; Brueckl, T.; Baxter, R. D.; Fujiwara, Y.; Seiple, I. B.; Su, S.; Blackmond, D. G.; Baran, P. S. *Proc. Natl. Acad. Sci. U.S.A.* **2011**, *108*, 14411–14415.

(389) Li, X.-G.; Dall'Angelo, S.; Schweiger, L. F.; Zanda, M.; O'Hagan, D. *Chem. Commun.* **2012**, *48*, 5247–5249.

(390) Dall'Angelo, S.; Bandaranayaka, N.; Windhorst, A. D.; Vugts, D. J.; Van der Born, D.; Onega, M.; Schweiger, L. F.; Zanda, M.; O'Hagan, D. *Nucl. Med. Biol.* **2013**, *40*, 464–470.

(391) Kularatne, S. A.; Wang, K.; Santhapuram, H.-K. R.; Low, P. S. *Molecular Pharmaceutics* **2009**, *6*, 780–789.

(392) Banerjee, S. R.; Foss, C. A.; Castanares, M.; Mease, R. C.; Byun, Y.; Fox, J. J.; Hilton, J.; Lupold, S. E.; Kozikowski, A. P.; Pomper, M. G. *J. Med. Chem.* **2008**, *51*, 4504–4517.

(393) Nitromed 2005, p. WO2005/30135 A2.

(394) Greenwald, R. B.; Choe, Y. H.; Conover, C. D.; Shum, K.; Wu, D.; Royzen, M. *J. Med. Chem.* **2000**, *43*, 475–487.

(395) Codelli, J. A.; Baskin, J. M.; Agard, N. J.; Bertozzi, C. R. *J. Am. Chem. Soc.* **2008**, *130*, 11486–11493.

(396) Still, W. C.; Kahn, M.; Mitra, A. *J. Org. Chem.* **1978**, *43*, 2923–2925.

(397) Armarego, W. L. F.; Chai, C. L. L. *Purification of Laboratory Chemicals*; 6th ed.; Butterworth-Heinemann, 2009.

(398) Douglas, A. W. *Can. J. Chem.* **1978**, *56*, 2129–2133.

(399) Bahrami, K.; Khodaei, M. M.; Khedri, M. *Chem. Lett.* **2007**, *36*, 1324–1325.

(400) Sakai, H.; Ito, K.; Sekiya, M. *Chem. Pharm. Bull.* **1873**, *21*, 2257–2264.

(401) Mukaiyama, T.; T., T.; Watanabe, K. *Chem. Lett.* **1978**, *7*, 1057–1060.

(402) Okabe, M.; Sun, R.-C.; Scalone, M.; Jibilian, C. H.; Hutchings, S. D. *J. Org. Chem.* **1995**, *60*, 767–771.

(403) Barton, D. H. R.; Cox, J. D. *J. Chem. Soc.* **1948**, 783–793.

(404) Miyaoka, H.; Tamura, M.; Yamada, Y. *Tetrahedron* **2000**, *56*, 8083–8094.

(405) Hu, M.; Li, L.; Wu, H.; Su, Y.; Yang, P.-Y.; Uttamchandani, M.; Xu, Q.-H.; Yao, S. Q. *J. Am. Chem. Soc* **2011**, *133*, 12009–12020.

(406) NICOX **2011**, WO2011/29065 A1 p76.

(407) Colombo, R.; Mingozzi, M.; Belvisi, L.; Arosio, D.; Piarulli, U.; Carenini, N.; Perego, P.; Zaffaroni, N.; De Cesare, M.; Castiglioni, V.; Scanziani, E.; Gennari, C. *J. Med. Chem.* **2012**, *55*, 10460–10474.

(408) Buechi, G.; Roberts, E. C. *J. Org. Chem.* **1968**, *33*, 460–462.

(409) Liu, F.; Zha, H.-Y.; Yao, Z.-J. *J. Org. Chem.* **2003**, *68*, 6679–6684.

(410) Jung, M. E.; Rohloff, J. C. *J. Org. Chem.* **1985**, *50*, 4909–4913.

(411) Wünsch, E.; Fries, G.; Zwick, A. *Chem. Ber.* **1958**, *91*, 542–547.

(412) Ross, A. J.; Lang, H. L.; Jackson, R. F. W. *J. Org. Chem.* **2009**, *75*, 245–248.

(413) Gerisch, S.; Jakubke, H.-D.; Kreuzfeld, H.-J. *Tetrahedron: Asymmetry* **1995**, *6*, 3039–3045.

(414) Szymańska, A.; Wegner, K.; Łankiewicz, L. *Helv. Chim. Acta* **2003**, *86*, 3326–3331.

(415) Couturier, C.; Blanchet, J.; Schlama, T.; Zhu, J. *Org. Lett.* **2006**, *8*, 2183–2186.

(416) Ikegame, F.; Yamamoto, A.; Sekine, T.; Ishikawa, T.; Kusame-Eguchi, K.; Kusama, T.; Watanabe, K. *Chem. Pharm. Bull.* **2000**, *48*, 278–280.

(417) Strazzolini, P.; Melloni, T.; Giumanini, A. G. *Tetrahedron* **2001**, *57*, 9033–9043.

(418) ChemImpex Int. *Catalogue #: 12219, CAS: 42417-76-5*.

(419) Schwyzer, R.; Iselin, B.; Kappeler, H.; Riniker, B.; Rittel, W.; Zuber, H. *Helv. Chim. Acta* **1963**, *46*, 1975–1996.

(420) Xia, Z.; Smith, C. D. *J. Org. Chem.* **2001**, *66*, 5241–5244.

(421) Zervas, L.; Winitz, M.; Greenstein, J. P. *J. Org. Chem.* **1957**, *22*, 1515–1521.